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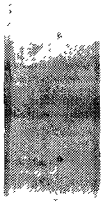

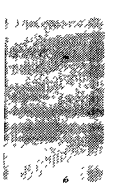

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DELMONT LABORATORIES, INC.
BIOLOGICAL SPECIALTIES
P. O. BOX AA, SWARTHMORE, PENNSYLVANIA 19081, U.S.A.

January 8, 1978

Miss Jennie C. Peterson
Hearing Clerk (HFC-20)
Food and Drug Administration
Room 4-65
5600 Fishers Lane
Rockville, Maryland 20857

Re: Docket No. 77N-0091 -- Bacterial Vaccines and Bacterial Antigens with No U.S. Standard of Potency

Dear Miss Peterson:

On November 8, 1977, the Food and Drug Administration issued a proposal to amend the biologics regulations in response to the report of the Advisory Panel on Bacterial Vaccines and Bacterial Antigens with No U.S. Standard of Potency. (42 Fed. Reg. 58266.) In accordance with the procedures established under 21 C.F.R. § 601.25 for the review to determine that licensed biological products are safe, effective, and not misbranded under prescribed, recommended, or suggested conditions of use, the proposal was based on a review of data submitted to the Advisory Panel by license holders and other interested persons. Delmont Laboratories, Inc., submitted data to the Panel to support the safety and effectiveness of its product Staphage Lysate (SPL) for Staphylococcal Disease.

On the basis of the Panel's recommendation, FDA has proposed to classify Staphage Lysate (SPL) in Category IIIB -- the category of products for which further testing is required to establish safety or effectiveness, but for which further marketing is not to be permitted. That recommendation was based on an assessment of the present evidence of safety and effectiveness of the product and the potential benefits and risks likely to result from the continued use of the product for a limited period while questions raised concerning the product are being resolved by further study. Since making its submissions to the Panel, Delmont has obtained important new information about the safety and effectiveness of SPL that supports a risk-benefit assessment favorable to inclusion of the product in Category IIIA. That information consists of:

1. Acute, subacute, and chronic toxicity studies of SPL in rats conducted by the Fujizoki Pharmaceutical Co., Ltd., of Tokyo, Japan. English translations of reports of those studies, which were sent to Delmont on November 29, 1977, are attached to these comments. (Exhibit 1.)

2. A teratogenicity study of SPL in rats, also conducted by the Fujizoki Pharmaceutical Co., Ltd. The report of that study, also sent to Delmont on November 29, is attached. (Exhibit 1.)

3. New evidence of the effectiveness and mode of action of SPL, contained in a report from Dr. Kenji Takeya, Professor of Bacteriology and President of Kyushu University in Fukuoka, Japan, sent to Delmont on December 1, 1977. The report shows that SPL treatment of mice previously sensitized with Stapylococcus aureus has a protective effect against herpes simplex virus inoculation. (Exhibit 2.)

4. A series of reports sent to Delmont by Fujizoki Pharmaceutical Co., Ltd., on December 28, 1977, dealing with specifications for SPL and assessment of its effectiveness as an immunopotentiator. Copies are attached. (Exhibit 3.)

In addition to these reports, Delmont has arranged for the conduct of a study based on short- and long-term surveillance of patients receiving Staphage Lysate therapy under the care of Arthur G. Baker, M.D. A protocol for that study is also attached to these comments. (Exhibit 4.) Results from the study should be available for reporting to FDA in late February or early March, 1978.

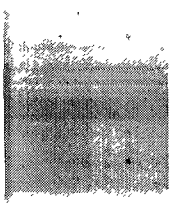
Delmont believes that these reports provide a more than adequate basis for revising the risk-benefit assessment made by FDA in its November 8 proposal. They show that no risk to human safety can result from continued marketing of SPL for a limited period while further studies are conducted, and they demonstrate that further studies of SPL in accordance with FDA requirements for clinical investigations will very likely provide substantial evidence that the product is effective for its labeled indications (although the mode of action through which it has its effect may differ from that which was postulated at one time). On the basis of this information, Delmont Laboratories urges that FDA reclassify

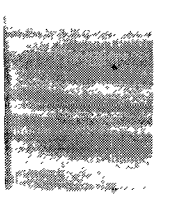
Staphage Lysate in Category IIIA to permit continued marketing pending completion of required studies.

Respectfully submitted,

Charles E. Lincoln RAK
Charles E. Lincoln
President

sw
Attachments
cc: John J. Singleton (HFB-620)





FUJIZOKI PHARMACEUTICAL CO.,LTD.

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Tokyo, November 29, 1977

Mr. Charles E. Lincoln, President
DELMONT LABORATORIES, INC.
P.O. Box AA, Swarthmore,
Pennsylvania 19081
U. S. A.

Dear Charlie:

We have heard the information of SPL from Dr. Aoki.

Enclosed please find the copies of "Acute and Subacute Toxicity Tests of SPL, Chronic Toxicity Test of SPL in rats and Teratologenicity Study of SPL in Rats.

Thanking you for your kind cooperation, we remain

Sincerely yours,

FUJIZOKI PHARMACEUTICAL CO., LTD

Junko Shiraishi
International Division

Chronic Toxicity Test of SPL in rats

Ryuichi FUJINO[1], Yuji SUGISAKI[1], Junko NAKAGAWA[1],
Masana KOMATSU[1]
and
Hachihiko HIRAYAMA[2]

Resarch Department Lab. I Fujizoki Pharmaceutical Co., Ltd.
6-7, Shimoochiai 4-chome, Shinjuku-ku, Tokyo[1]

Resarch Department Lab. II Fujizoki Pharmaceutical Co., Ltd.
9-14, Nishiki 2-chome, Nerima-ku, Tokyo[2]

Summary

Chronic toxicity of SPL for staphylococcal disease manufactured by Delmont Laboratories Inc. was investigated in comparison with sterile saline as a control group for 182 days in Wistar rats.

Rats were given once a day at a subcutaneous dose of 0.8, 4.0 and 20.0 ml/kg of SPL and 20.0 ml/kg of sterile saline, respectively.

They were observed daily for appearance, behavior and survival: body weights were mesured periodically. All animals were sacrificed and examined at necropsy for abnormalities, and subsequently for histomorphologic alterations. Heamotological, biochemical and urinary examinations performed respectively.

No remarkable changes of every observations were observed in all groups treated with SPL and control group.

Acute and Subacute Toxicity Tests of SPL

Ryuichi FUJINO, Yuji SUGISAKI, Junko NAKAGAWA
and
Masana KOMATSU
Resarch Department Lab. I
Fujizoki Pharmaceutical Co., Ltd.
6-7, Shimoochiai 4-chome,
Shinjuku-ku, Tokyo

Acute and Subacute toxicities of SPL for stuphylococcal disease manufactured by Delmont Laboratories Inc. were studied in mice and rats.

LD_{50} of SPL were as follows:

Species	Sex	Administration route (ml/kg)			
		p.o.	s.c.	i.p.	i.v.
Mouse	Male	100<	100<	100<	100<
	Female	100<	100<	100<	100<
Rat	Male	50<	50<	70<	50<
	Female	50<	50<	70<	50<

The median lethal doses for every administration routes in mice and rats were more than technically applicable maximum doses. In subacute toxicity test for 30 days, rats were given once a day subcutaneous dose of 2.0, 7.0 and 20.0 ml/kg, respectively. The control group recieved sterile saline at 20.0 ml/kg. They were observed daily for appearance, behavior and survival; body weights were measured periodically. All animals were sacrificed and examined at necropsy for abnormalities, and subsequently for histomorpholagic alterations. Hematological, biochemical and urinary examinations performed respectively.

No remarkable changes of every observations were observed in all groups treated with SPL and control group.

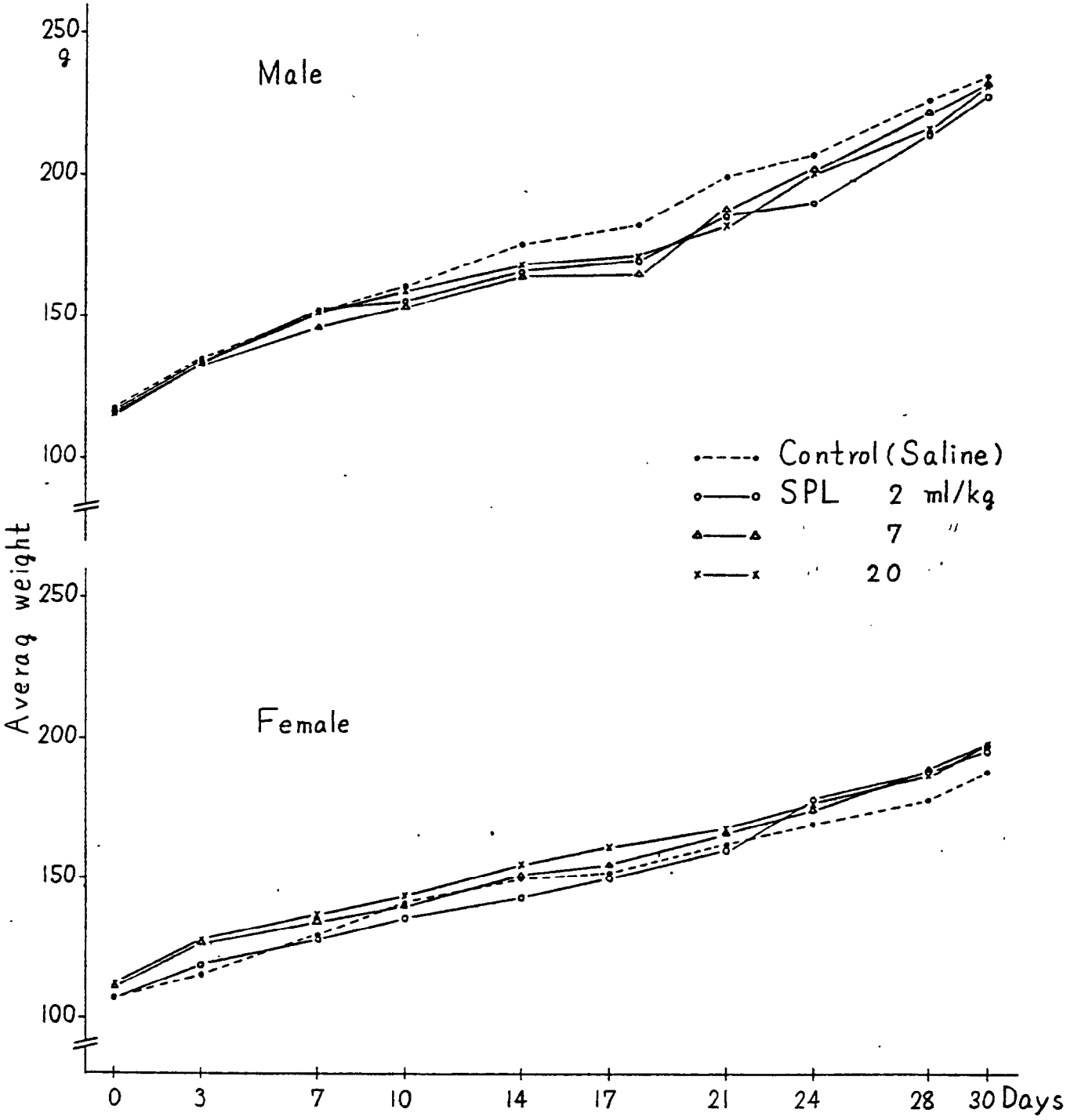


Fig. 1 Average body weights in male and female rats treated with SPL (s.c) for 30 days

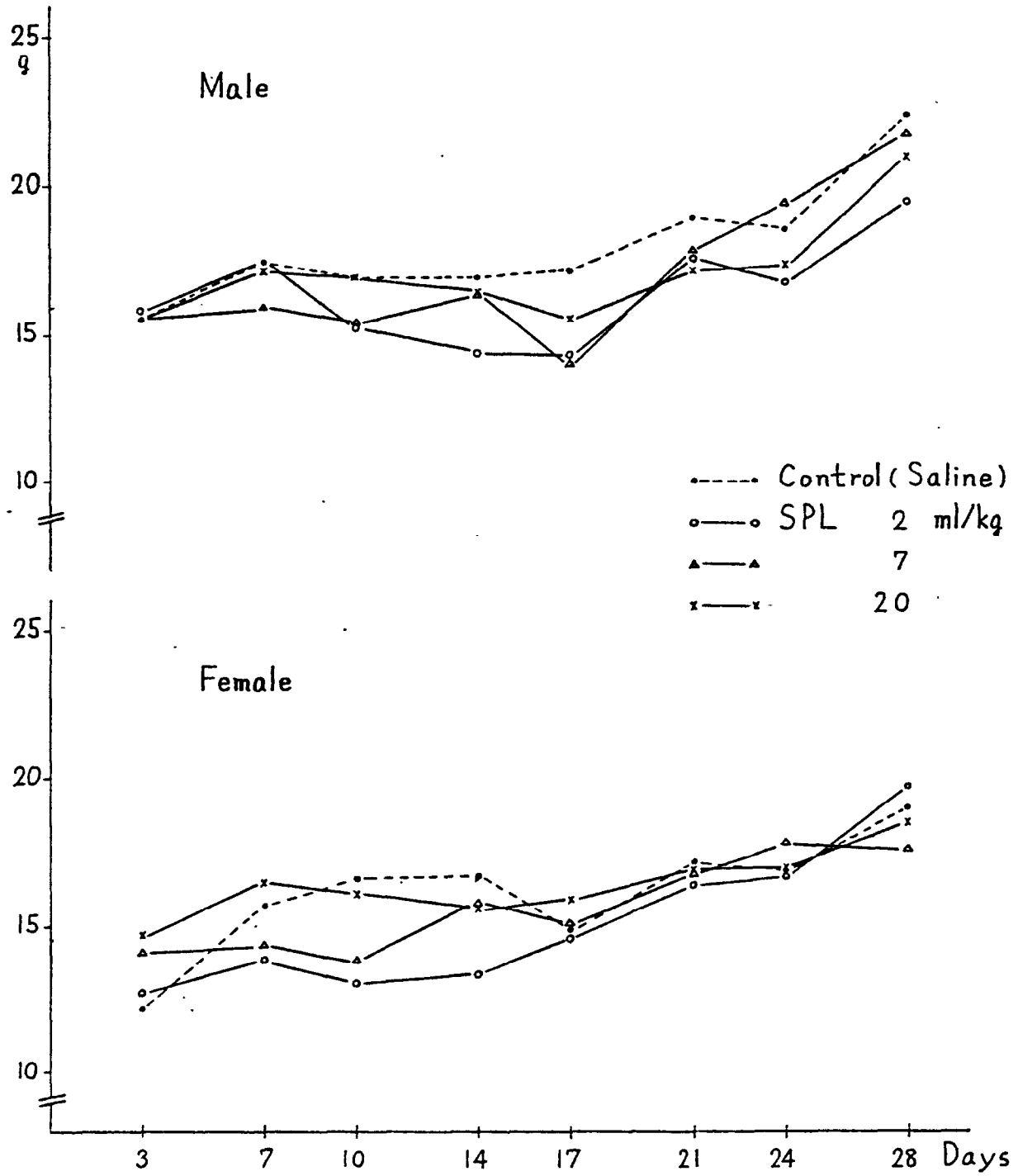


Fig. 2 Food consumptions in male and female rats treated with SPL (s.c) for 30 days

Table 1 Average wet weight of various organs in rats treated with SPL (s.c.) for 30 days (Mean ± S.D.)

Tissue	Sex	Control (Saline)	SPL		
			2 ml/kg	7 ml/kg	20 ml/kg
Body weight (g)	M	234 ±34	227 ±45	232 ±43	231 ±31
	F	188 ±13	195 ±17	198 ±19	198 ±21
Brain (g)	M	1.75± 0.11	1.77± 0.09	1.78± 0.19	1.81± 0.10
	F	1.64± 0.05	1.62± 0.07	1.62± 0.04	1.62± 0.09
Lung (g)	M	2.24± 1.58	2.13± 0.63	1.96± 0.62	1.96± 0.44
	F	1.51± 0.46	1.55± 0.51	1.60± 0.26	1.32± 0.25
Thymus (g)	M	0.69± 0.16	0.65± 0.17	0.71± 0.17	0.61± 0.18
	F	0.52± 0.09	0.57± 0.09	0.55± 0.10	0.50± 0.06
Heart (g)	M	0.95± 0.13	0.99± 0.18	0.98± 0.23	0.96± 0.12
	F	0.74± 0.04	0.71± 0.04	0.74± 0.08	0.75± 0.08
Liver (g)	M	11.81± 1.76	10.65± 2.76	11.05± 1.93	10.77± 1.44
	F	9.91± 1.32	9.52± 1.64	9.62± 1.23	9.53± 1.35
Kidneys (g)	M	2.20± 0.39	2.26± 0.55	2.45± 0.70	2.49± 0.34
	F	1.79± 0.12	1.73± 0.19	1.74± 0.15	1.67± 0.20
Spleen (g)	M	0.69± 0.08	0.61± 0.20	0.70± 0.15	0.74± 0.17
	F	0.64± 0.09	0.62± 0.20	0.54± 0.05*	0.60± 0.09
Adrenals (mg)	M	58.4 ± 8.2	54.7 ± 10.3	54.1 ± 8.2	53.8 ± 4.9
	F	61.5 ± 6.0	53.9 ± 6.6*	56.7 ± 10.1	58.0 ± 9.3
Thyroid (mg)	M	35.9 ± 9.5	38.8 ± 10.2	38.2 ± 8.5	37.1 ± 7.2
	F	33.2 ± 6.0	32.0 ± 5.5	32.6 ± 4.0	33.1 ± 3.1
Hypophysis (mg)	M	12.3 ± 4.7	12.5 ± 4.9	11.4 ± 3.8	11.3 ± 2.3
	F	13.3 ± 3.5	13.1 ± 4.0	13.4 ± 2.6	11.1 ± 3.9
Testes (g)	M	2.32± 0.44	2.21 ± 0.32	2.29± 0.26	2.24± 0.30
Seminal vesicle (g)	M	0.67± 0.24	0.82± 0.41	0.60± 0.45	0.54± 0.31
Ovaries (mg)	F	73.5 ± 13.6	67.8 ± 12.0	68.9 ± 7.9	69.3 ±12.7
Uterus (g)	F	0.34 ± 0.08	0.34 ± 0.05	0.32± 0.05	0.37± 0.08

M: Male F: Female

*: $p < 0.05$

Table 2 Average wet weight of various organs (/100g body weight) in rats treated with SPL (s.c.) for 30 days (Mean ± S.D.)

Tissue	Sex	Control (Saline)	SPL		
			2 ml/kg	7 ml/kg	20 ml/kg
Brain	M	0.76 ± 0.09	0.80 ± 0.16	0.78 ± 0.10	0.80 ± 0.10
(g)	F	0.88 ± 0.06	0.84 ± 0.05	0.83 ± 0.07	0.82 ± 0.05
Lung	M	1.06 ± 1.05	0.99 ± 0.41	0.92 ± 0.50	0.88 ± 0.30
(g)	F	0.82 ± 0.31	0.79 ± 0.26	0.82 ± 0.18	0.68 ± 0.19
Thymus	M	0.29 ± 0.04	0.29 ± 0.05	0.31 ± 0.03	0.26 ± 0.06
(g)	F	0.28 ± 0.04	0.29 ± 0.03	0.28 ± 0.04	0.25 ± 0.00
Heart	M	0.41 ± 0.06	0.44 ± 0.03	0.43 ± 0.04	0.42 ± 0.00
(g)	F	0.39 ± 0.00	0.36 ± 0.00	0.38 ± 0.03	0.38 ± 0.03
Liver	M	5.07 ± 0.47	4.63 ± 0.44	4.79 ± 0.30	4.68 ± 0.35
(g)	F	5.26 ± 0.51	4.89 ± 0.88	4.86 ± 0.39	4.82 ± 0.50
Kidneys	M	0.95 ± 0.16	0.99 ± 0.10	1.05 ± 0.18	1.08 ± 0.14
(g)	F	0.96 ± 0.09	0.89 ± 0.05	0.88 ± 0.04*	0.85 ± 0.10*
Spleen	M	0.30 ± 0.05	0.27 ± 0.04	0.31 ± 0.04	0.32 ± 0.07
(g)	F	0.34 ± 0.03	0.32 ± 0.11	0.27 ± 0.00	0.30 ± 0.03
Adrenals	M	25.4 ± 4.8	24.7 ± 5.6	23.8 ± 4.2	23.7 ± 4.0
(mg)	F	32.8 ± 2.9	27.6 ± 2.4***	29.2 ± 4.9	29.3 ± 3.5*
Thyroid	M	15.3 ± 3.2	17.0 ± 2.4	16.7 ± 3.1	16.1 ± 2.6
(mg)	F	17.6 ± 2.7	16.5 ± 3.0	16.6 ± 2.5	16.9 ± 2.3
Hypophysis	M	5.5 ± 2.4	5.5 ± 2.3	5.1 ± 2.0	5.0 ± 1.2
(mg)	F	7.1 ± 1.8	6.7 ± 1.9	6.8 ± 1.4	5.5 ± 2.0
Testes	M	0.99 ± 0.12	1.00 ± 0.18	1.01 ± 0.13	0.97 ± 0.10
(g)					
Seminal vesicle	M	0.28 ± 0.08	0.34 ± 0.15	0.24 ± 0.14	0.23 ± 0.10
(g)					
Ovaries					
(mg)	F	39.1 ± 6.6	34.7 ± 5.8	34.9 ± 3.2	35.0 ± 4.9
Uterus					
(g)	F	0.18 ± 0.03	0.17 ± 0.00	0.16 ± 0.00	0.20 ± 0.04

M: Male F: Female

*: $P < 0.05$ ***: $P < 0.001$

Table 3 Biochemical examination on serum in rats treated with SPL.(s.c.) for 30 days (Mean ± S.D.)

	Sex	Control (Saline)	SPL 2 ml/kg	SPL 7 ml/kg	SPL 20 ml/kg
Total bilirubin (mg/dl)	M	0.2± 0.0	0.2± 0.0	0.2± 0.0	0.2± 0.0
	F	0.2± 0.0	0.2± 0.0	0.2± 0.0	0.2± 0.0
Thymol turbidity test (Maclagan unit)	M	0.4± 0.1	0.4± 0.2	0.4± 0.1	0.4± 0.2
	F	0.3± 0.1	0.3± 0.1	0.3± 0.1	0.3± 0.1
Alkaline phosphatase (Kind-King unit)	M	20.2± 9.6	18.7± 3.5	19.8± 5.7	17.6± 1.8
	F	12.7± 2.7	13.4± 2.9	12.2± 2.1	12.3± 2.0
S-GOT (Karmen unit)	M	180 ±51	172 ±40	171 ±21	165 ±22
	F	140 ±17	170 ±55	158 ±23	135 ±18
S-GPT (Karmen unit)	M	44 ±12	48 ±10	43 ± 5	40 ± 8
	F	49 ±15	44 ±15	51 ±19	54 ±20
Total protein (g/dl)	M	6.3± 0.3	6.2± 0.4	6.2± 0.4	6.2± 0.3
	F	6.6± 0.4	6.4± 0.2	6.3± 0.3	6.6± 0.3
A/G ratio	M	0.9± 0.1	0.9± 0.1	1.0± 0.1	0.9± 0.1
	F	0.9± 0.1	0.9± 0.1	1.0± 0.1	0.9± 0.1
Albumin (g/dl)	M	3.0± 0.1	3.0± 0.2	3.0± 0.1	3.0± 0.1
	F	3.1± 0.1	3.1± 0.2	3.1± 0.1	3.2± 0.1
Total cholesterol (mg/dl)	M	51 ± 9	50 ±15	55 ± 5	55 ±10
	F	49 ± 5	45 ±10	43 ± 5	48 ± 5
Blood urea nitrogen (mg/dl)	M	23 ± 3	21 ± 5	23 ± 4	23 ± 3
	F	22 ± 4	21 ± 4	21 ± 2	20 ± 2
Creatinine (mg/dl)	M	0.7± 0.0	0.7± 0.1	0.6± 0.0	0.7± 0.1
	F	0.7± 0.1	0.7± 0.1	0.7± 0.1	0.7± 0.0
Sodium mEq/l	M	141 ± 1	145 ±10	148 ±18	145 ± 4 *
	F	144 ± 8	142 ± 1	141 ± 2	140 ± 2
Potassium mEq/l	M	5.9± 0.9	5.9± 0.7	6.3± 0.3	6.2± 0.5
	F	5.7± 0.4	5.5± 0.5	5.3± 0.3 *	5.0± 0.4 ***
Blood suger mg/dl	M	172 ±26	175 ±12	149 ±18 *	159 ±19
	F	158 ±16	168 ±17	165 ±19	171 ±15

M: Male F: Female

*: $p < 0.05$ ***: $p < 0.001$

Table 4. Hematological findings in rats treated with SPL (s.c.) for 30 days (Mean ± S.D.)

		Sex	Control (Saline)	SPL 2 ml/kg	SPL 7 ml/kg	SPL 20 ml/kg
Hemoglobin (g/dl)		M	15.6 ± 0.7	16.6 ± 1.0	15.4 ± 0.6	15.2 ± 0.9
		F	15.3 ± 1.2	15.8 ± 0.8	15.8 ± 0.9	15.3 ± 0.6
Hematocrit (%)		M	45.8 ± 2.7	47.6 ± 5.5	45.7 ± 2.4	45.9 ± 3.3
		F	46.1 ± 2.9	45.0 ± 2.0	45.5 ± 1.7	45.0 ± 1.9
Erythrocytes ($\times 10^4$)		M	639 ±137	778 ±205	820 ±132*	784 ± 81*
		F	702 ±134	767 ± 97	720 ±157	807 ± 83
Leucocytes ($\times 10^2$)		M	88 ± 35	112 ± 39	87 ± 46	70 ± 15
		F	95 ± 21	107 ± 31	93 ± 17	111 ± 20
Hemogramm %	Baso.	M	0	0	0	0
		F	0	0	0	0
	Eosin.	M	0.3 ± 0.3	0.7± 0.4*	0.8± 0.4**	0.7 ± 0.4
		F	1.3 ± 0.6	1.2± 0.4	1.4± 1.0	1.5 ± 1.0
	Neut.	M	22.5 ± 12.8	23.3 ± 10.6	18.1 ± 7.3	21.0 ± 8.5
		F	20.8 ± 6.7	13.2 ± 5.1*	20.2 ± 5.3	18.4± 6.4
	Lymph.	M	74.1 ± 12.2	73.2 ± 10.3	78.0± 8.2	75.1 ± 8.4
		F	74.7 ± 7.0	81.3 ± 5.6	75.8 ± 5.4	76.3 ± 7.9
	Mono.	M	3.0 ± 0.8	2.8 ± 1.4	3.1 ± 1.3	3.2 ± 1.1
		F	3.2 ± 1.1	4.3 ± 2.3	2.6 ± 1.4	3.8 ± 1.8

M: Male F: Female

*: $p < 0.05$ **: $p < 0.01$

Table 5. Urinalysis in rats treated with SPL (s.c.) for 30 days

		Control (Saline)		SPL					
				2 ml/kg		7 ml/kg		20 ml/kg	
		M /9	F /10	M /9	F /9	M /8	F /10	M /8	F /9
pH	6	0	8	1	4	0	6	1	7
	7	7	2	8	5	8	4	7	2
	8	2	0	0	0	0	0	0	0
Protein	−	1	0	0	0	0	0	0	0
	±	6	9	8	9	5	10	8	9
	+	0	1	0	0	0	0	0	0
	++	2	0	1	0	3	0	0	0
Glucose	−	9	10	9	9	7	10	8	9
	±	0	0	0	0	1	0	0	0
Ketons	−	9	10	9	9	8	9	8	9
	±	0	0	0	0	0	1	0	0
Occult blood	−	9	7	6	7	6	7	6	6
	±	0	2	3	2	2	3	2	3
	+	0	1	0	0	0	0	0	0

M: Male F: Female

Teratologenicity Study of SPL in Rats and Rabbits

Hachihiko HIRAYAMA

Resarch Department Lab. II
Fujizoki Pharmaceutical Co., Ltd.
9-14, Nishiki 2-chome,
Nerima-ku, Tokyo

Summary

Teratologenicity study of SPL for staphylococcal disease manufactured by Delmont Laboratories Inc. was carried out in rats and rabbits. SPL was intraperitonially given to pregnant rats for 11 days from day 6 to day 16 of gestation at dose levels of 0.02, 0.5 and 5 ml/kg/day and to pregnant rabbits for 13 days from day 6 to day 18 of gestation at dose levels of 0.02, 0.2 and 2.0 ml/kg/day.

SPL had no teratologenic effect on both animals.

Table 1 Teratogenic effects of S - 27 against rat fetuses

	Control	S - 27 (ml/kg/day x 11 ; i.p.)		
		0.05	0.5	5.0
No. of dams	2	3	5	4
Total No. of implantation	25	29	48	38
No. of survival fetuses	24	28	44	38
(Mean litter size)	(12.0)	(9.3)	(8.8)	(9.5)
Dead or rfsorped fetuses	1	1	4	0
(Fetal mortality ; %)*	4.0	3.4	8.3	0
Sex ratio (♂/♀)	12/12	17/11	24/20	20/18
Mean fetal body weight (g)(♂)	3.51	3.51	3.39	3.45
(♀)	3.11	3.28	3.06	3.29
External anomalies	0	0	0	0
(%)**	(0)	(0)	(0)	(0)
Skeletal anomalies	0	0	0	0
(%)**	(0)	(0)	(0)	(0)
Visceral anomalies	0	0	0	0
(%)**	(0)	(0)	(0)	(0)
Growth retardation***	1	1	6	0
(%)**	(4.2)	(3.6)	(13.6)	(0)

* : No. of dead or resorped fetuses/ No. of total implantation x 100 (%)
** : No. of abnormal fetuses/ No. of fetuses examined x 100 (%)
*** : Body weight $<$3.0 g.

Table 2 Teratogenic effects of S - 27 against rabbit fetuses

	Control	S - 27 (ml/kg/day x 13 ; i.p.)		
		0.02	0.2	2.0
No. of dams	1	3	3	2
Total No. of implantation	7	24	18	16
No. of survival fetuses	6	20	18	14
(Mean litter size)	(6)	(6.3)	(6.0)	(6.5)
No. of dead or resorped fetuses	1	4	0	2
(Fetal mortality)*	(14.3)	(16.6)	(0)	(12.5)
Mean fetal body weight (g)	53.1	54.1	53.2	50.7
External anomalies	0	0	0	0
(%)*	(0)	(0)	(0)	(0)
Skeletal anomalies	0	0	0	0
(%)**	(0)	(0)	(0)	(0)
Visceral anomalies	0	0	0	0
(%)	(0)	(0)	(0)	(0)

* : No. of dead or resorped fetuses/ No. of total implantation x100 (%).

** : No. of abnormal fetuses/ No. of fetuses examined x 100 (%).

PHOTO-I

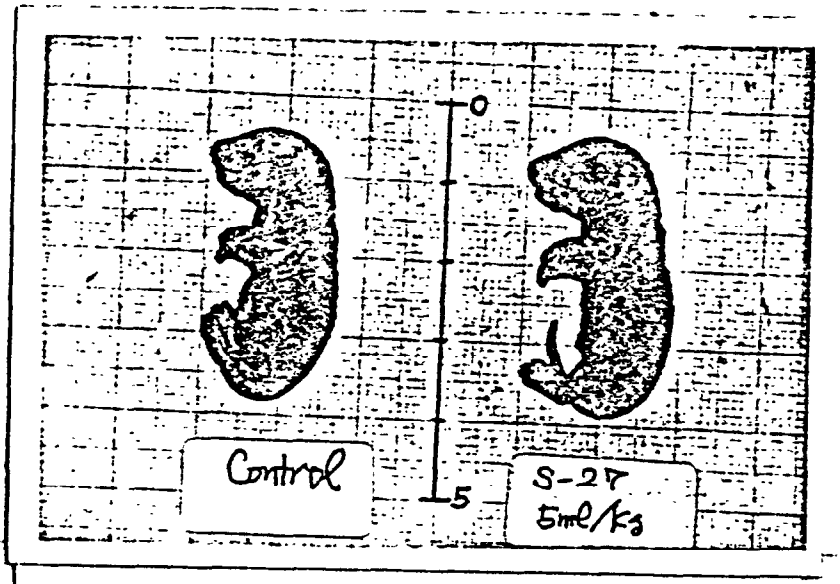


PLATE I-1 Left: A fetus from the pregnant rat. Right: A fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

PLATE I-2 A normal skeleton of rat fetus in saline control group.

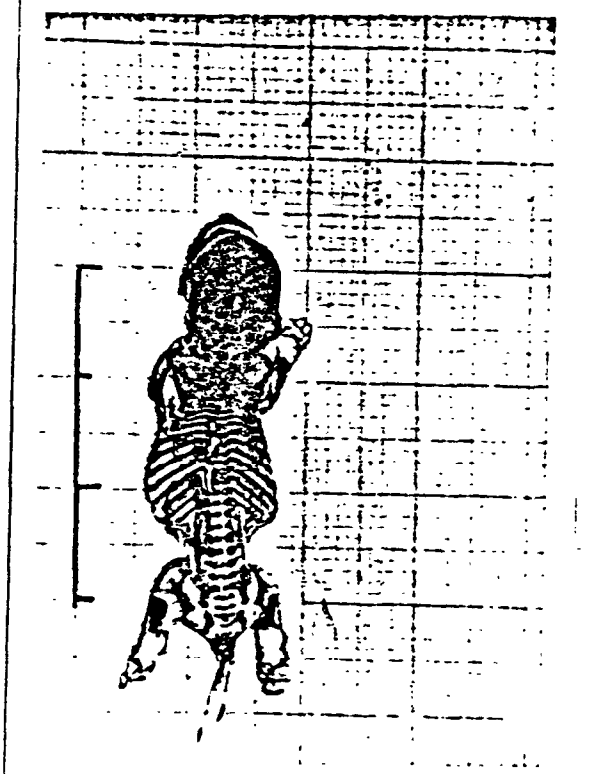


PLATE I-3 A normal skeleton of rat fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

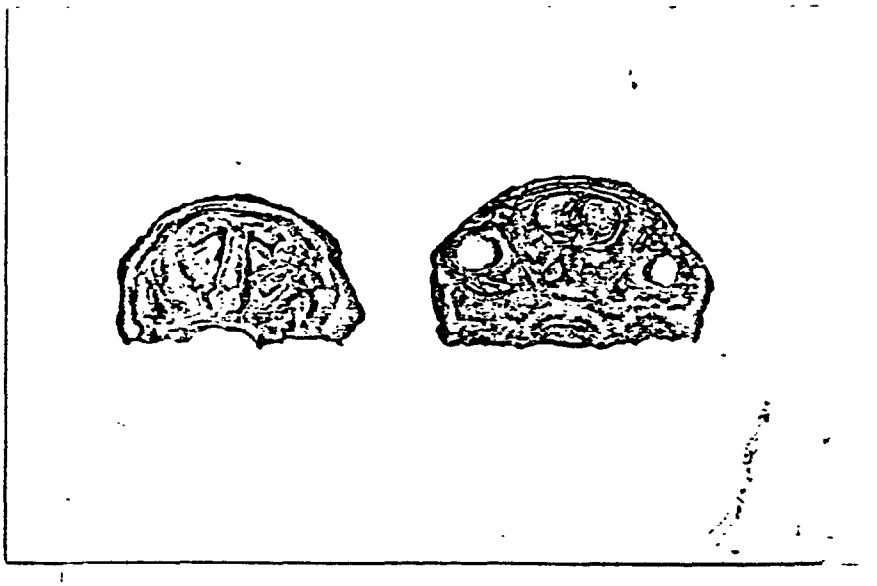


PLATE I-4 Palate and nasal cavities of control rat fetus; showing normally in the Wilson's section.

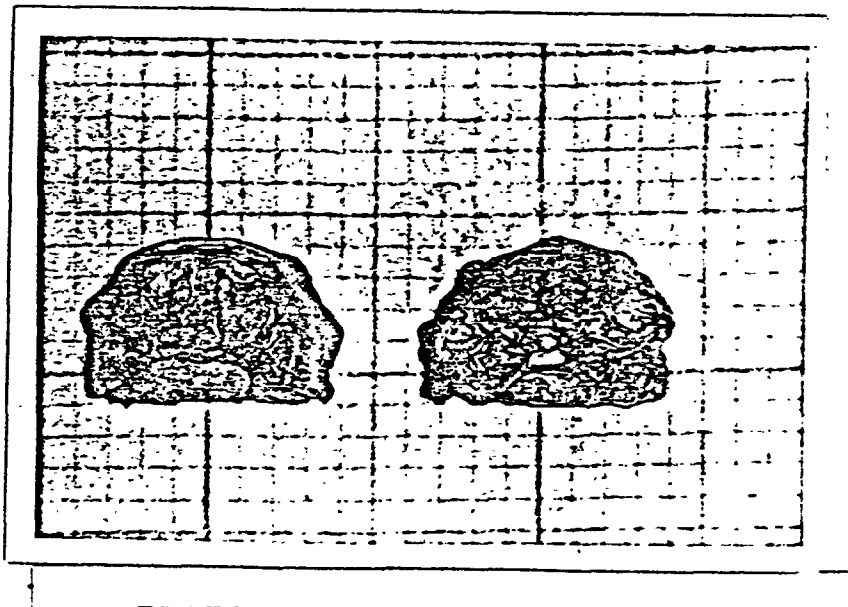


PLATE I-5 Palate, eyeballs and olfactory bulbs of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

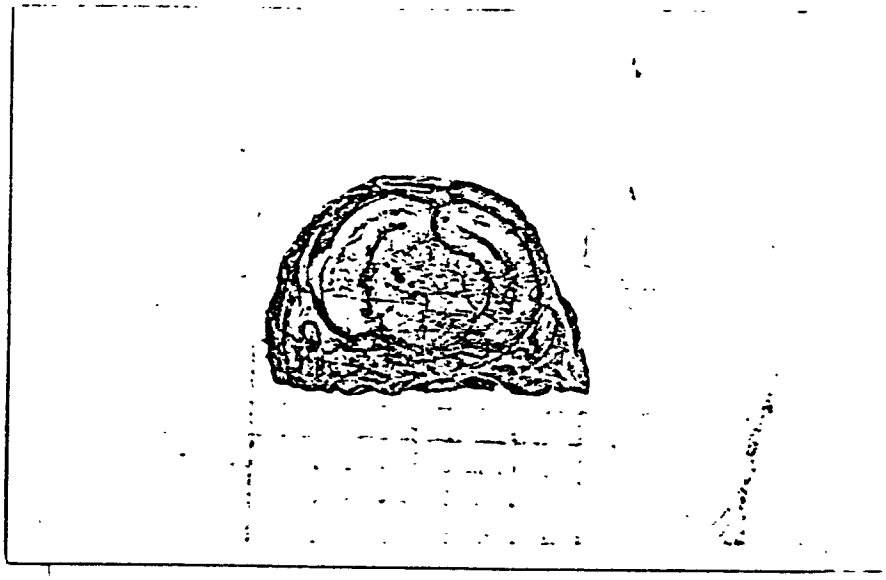


PLATE I-6 Skull and brain in control fetus; showing normally in the Wilson's section.

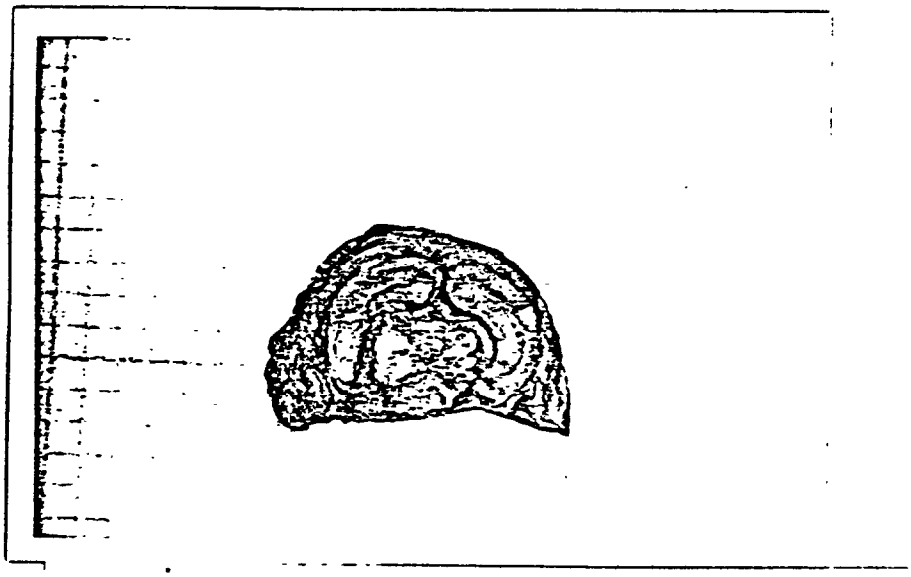


PLATE I-7 Skull and brain of fetus from the pregnant rat treated introperitoneally with 5ml/kg of S-27.

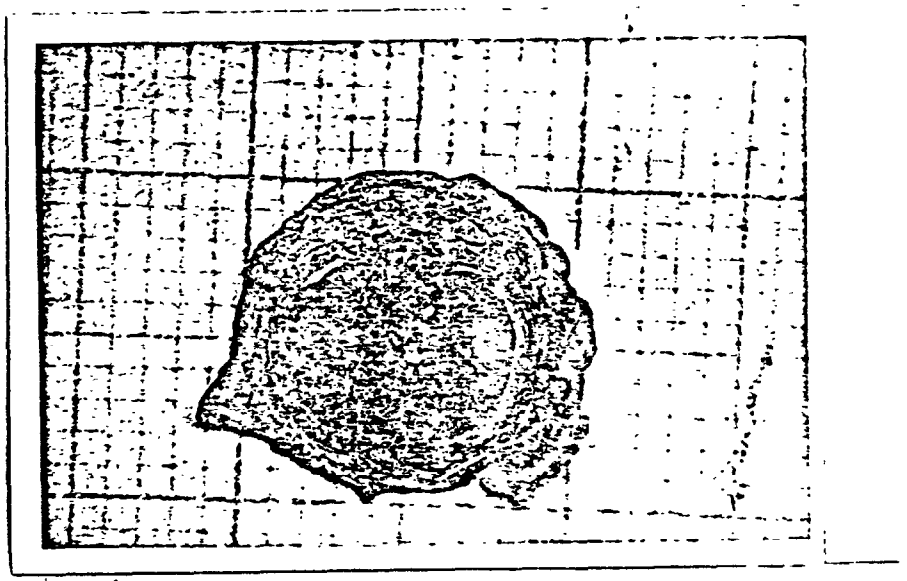


PLATE I-8 Cardiac ventricle and lung section of control fetus; showing normally in Wilson's section.

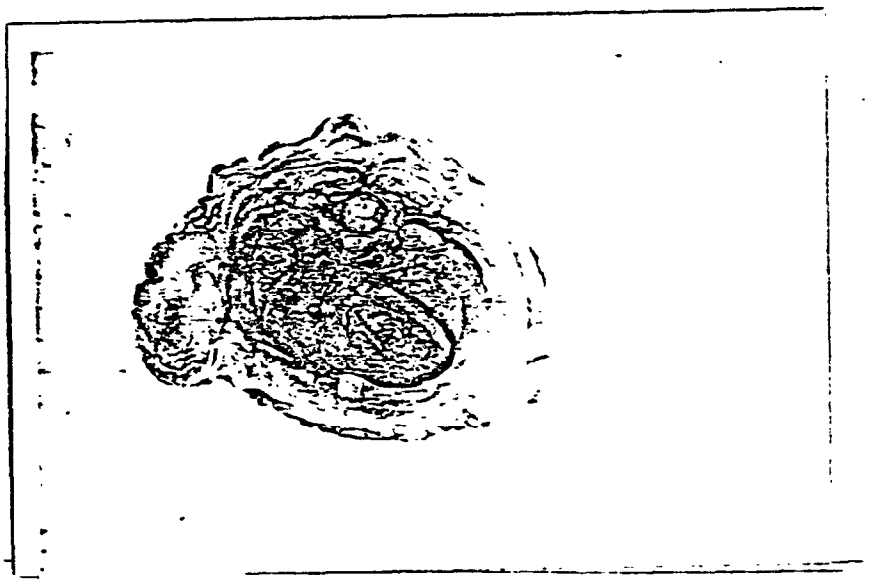


PLATE I-9 Cardiac ventricle, lung and heart of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27; showing normally in Wilson's section.

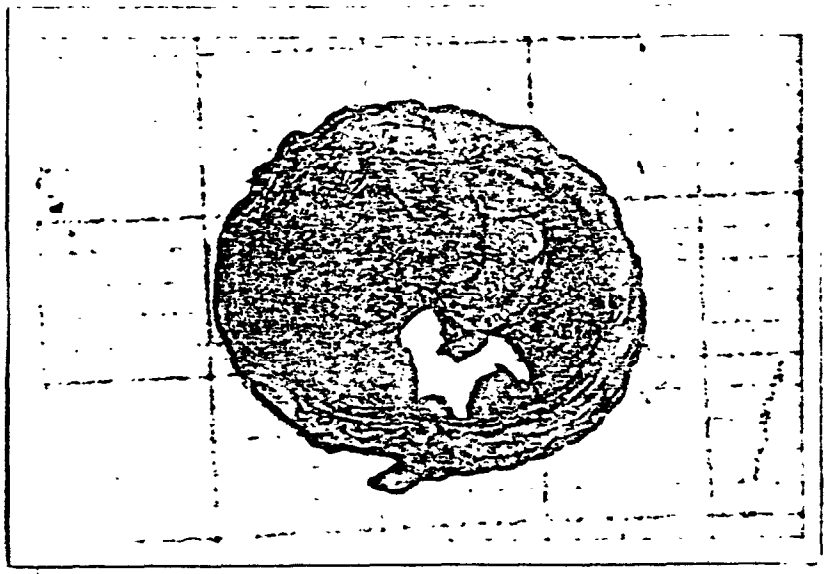


PLATE I-10 Liver ~~kidney~~ and stomach of control fetus; showing normally in the Wilson's section.

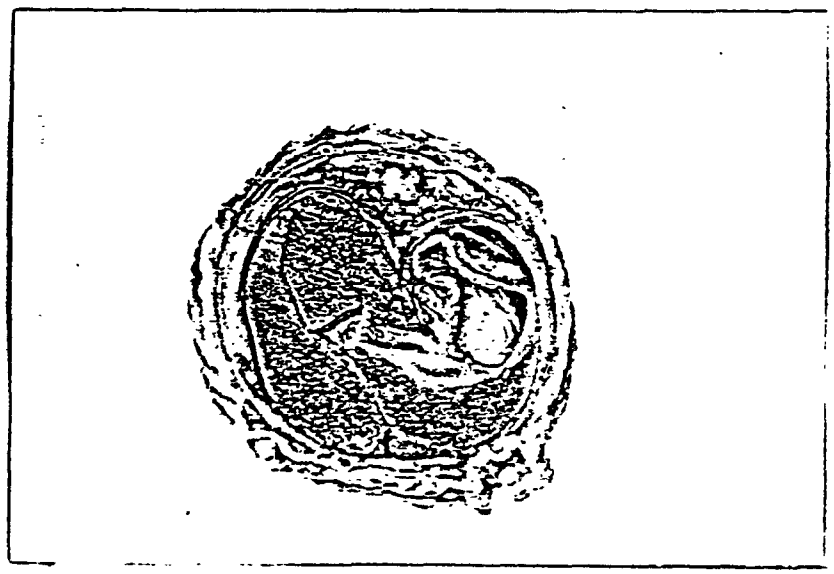


PLATE I-11 Liver and stomach of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27.

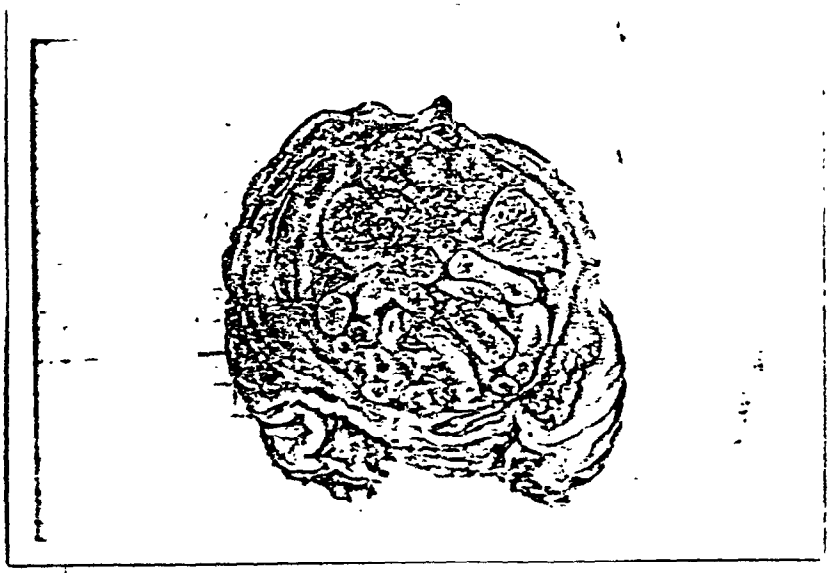


PLATE I-12 Kidney, intestine and spleen of control fetus; showing normally in the Wilson's section

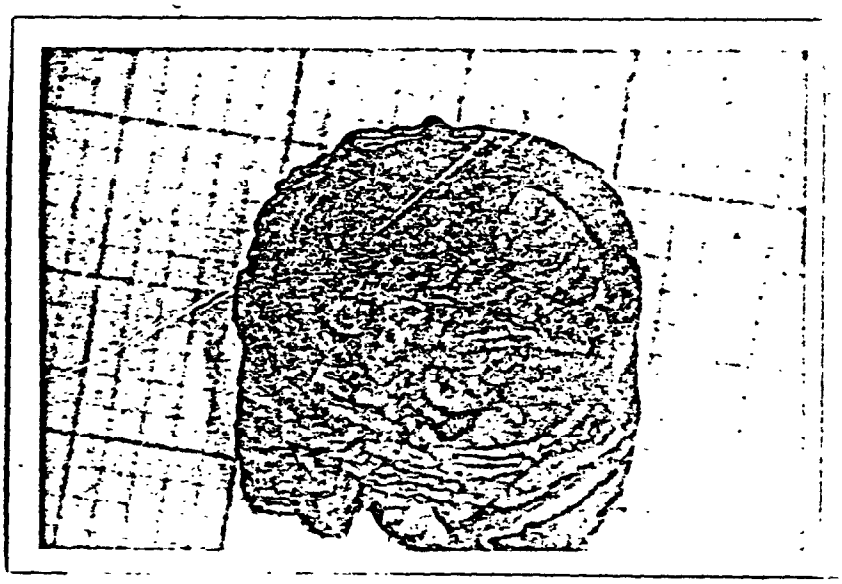


PLATE I-13 Kidney, testis and urinary bladder of control fetus; showing normally in the Wilson's section.

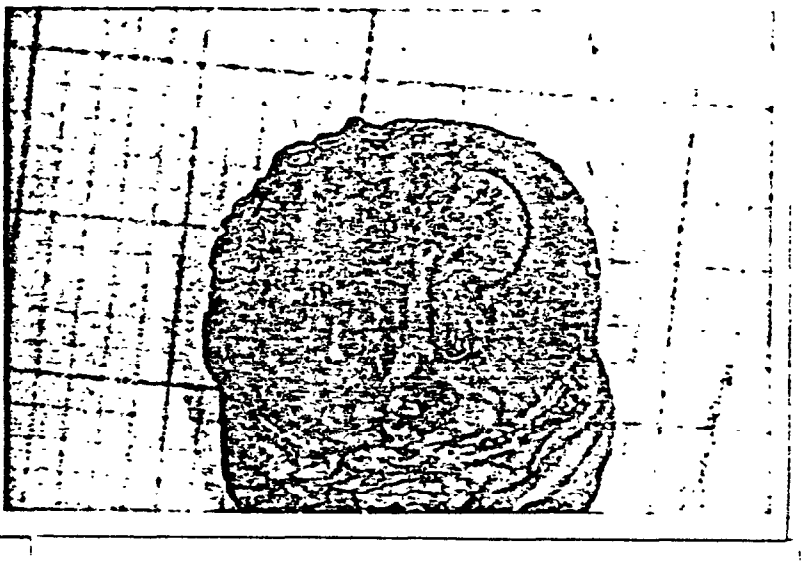


PLATE I-12 Kidney, intestine and spleen of control rabbit fetus; showing normally in the Wilson's section.

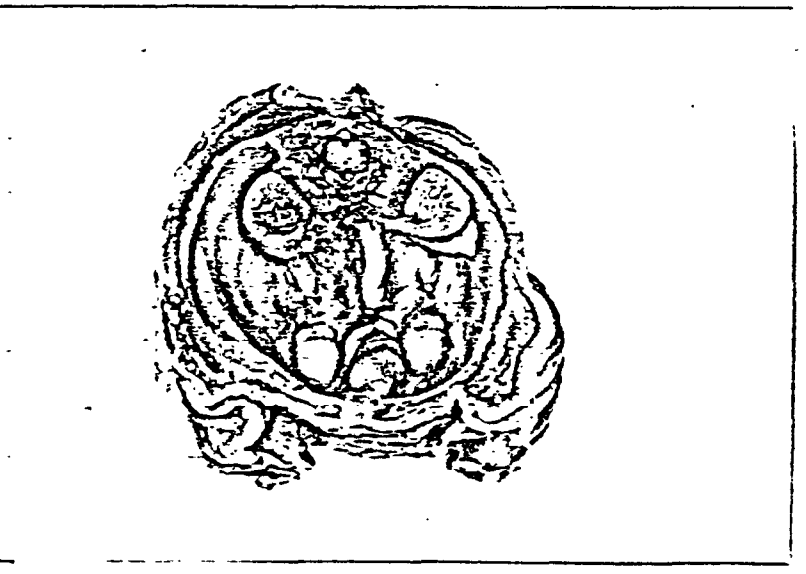


PLATE I-14 Kidney, testis and urinary bladder of fetus from the pregnant rat treated intra-peritneally with 5ml/kg of S-27; showing normally in the Wilson's section.

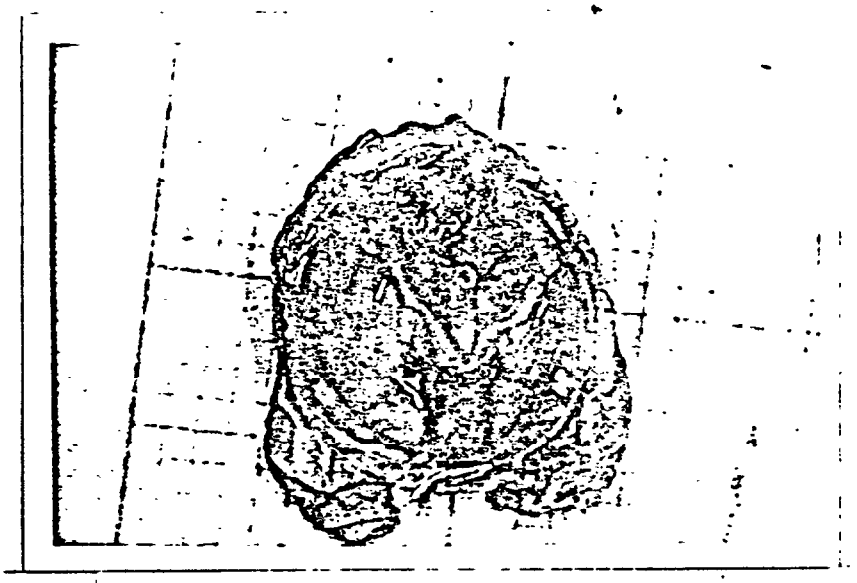


PLATE I-15 Kidney, ovary and uterus of control fetus; showing normally in the Wilson's section.

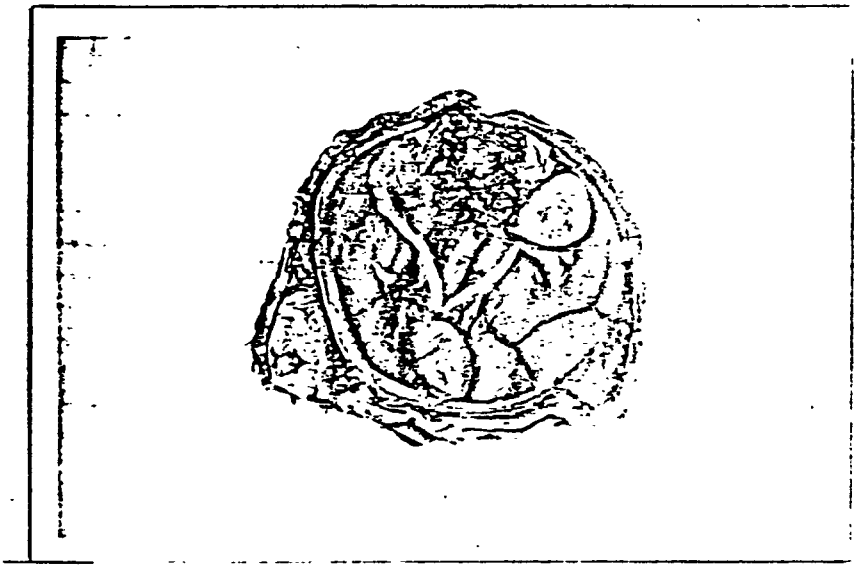


PLATE I-16 Kidney, ovary and uterus of fetus from the pregnant rat treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

PHOTO-II

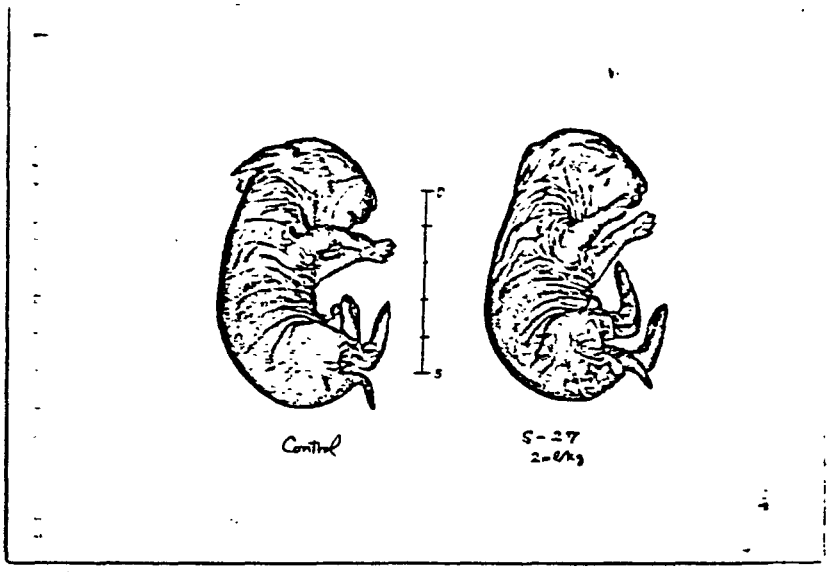


PLATE II-1 Left: A fetus from the pregnant rabbit. Right: A fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

PLATE II-2 A normal skeleton of rabbit fetus in saline control group.

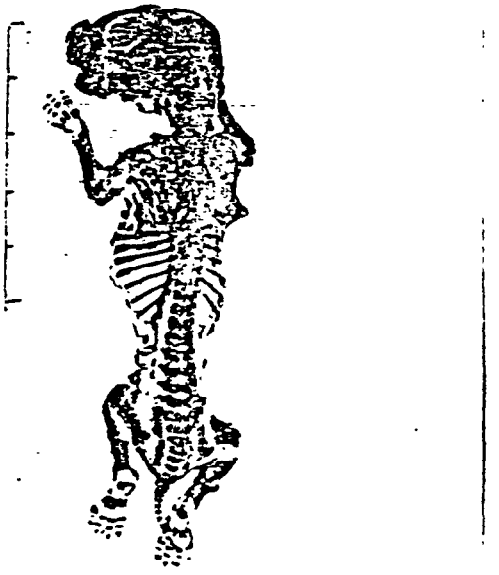


PLATE II-3 A normal skeleton of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

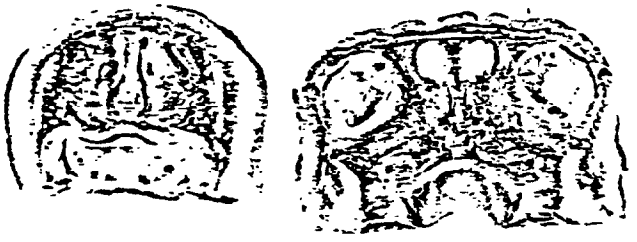


PLATE II-4 Palate and nasal cavities of control rabbit fetus; showing normally in the Wilson's section.

PLATE II-5 Palate, eyeballs and olfactory bulbs of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.

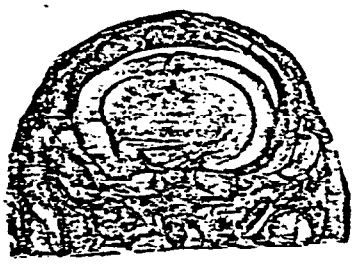


PLATE II-6 Skull and brain in control rabbit fetus; showing normally in the Wilson's section.

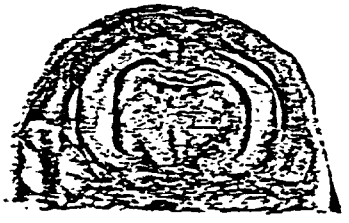


PLATE II-7 Skull and brain of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

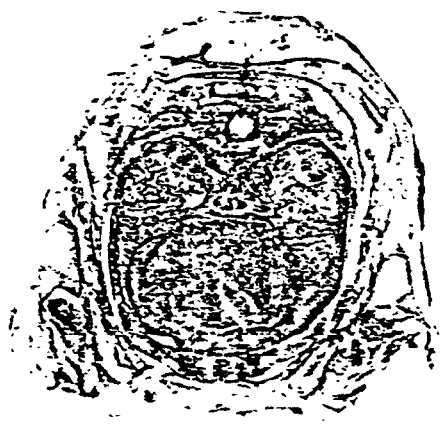


PLATE II-8 Cardiac ventricle and lung section of control rabbit fetus; showing normally in Wilson's section.

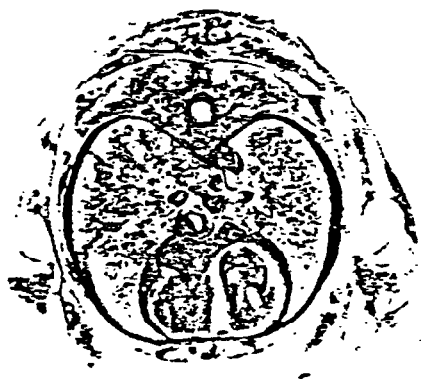


PLATE II-9 Cardiac ventricle, lung and heart of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in Wilson's section.

PLATE Ⅱ-10 Liver, kidney and stomach of control rabbit fetus; showing normally in the Wilson's section.

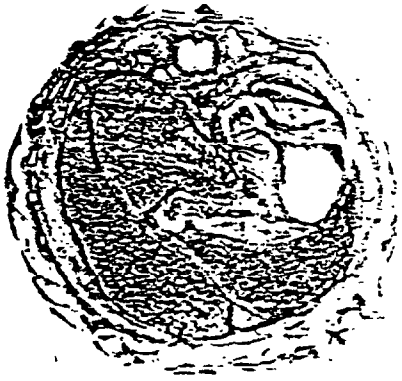


PLATE Ⅱ-11 Liver and stomach of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27.

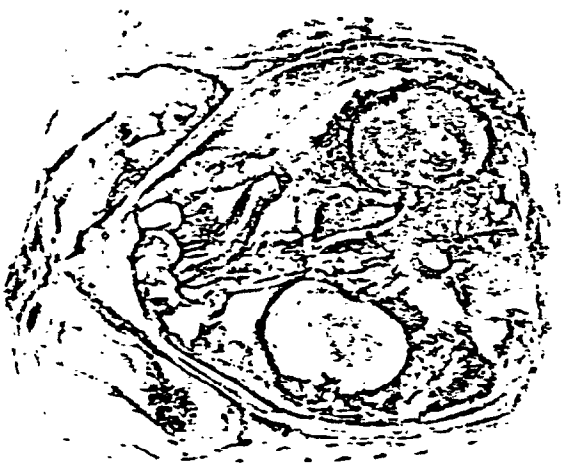


PLATE II-13 Kidney, testis and urinary bladder of control rabbit fetus; showing normally in the Wilson's section.

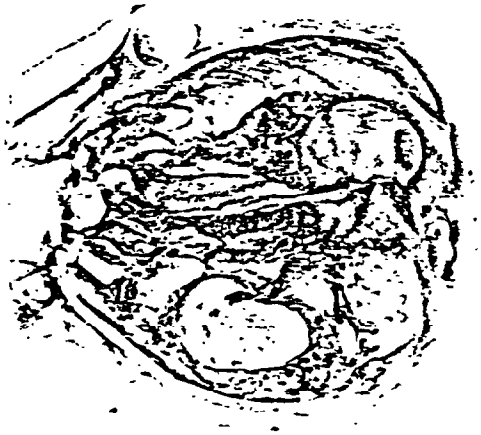
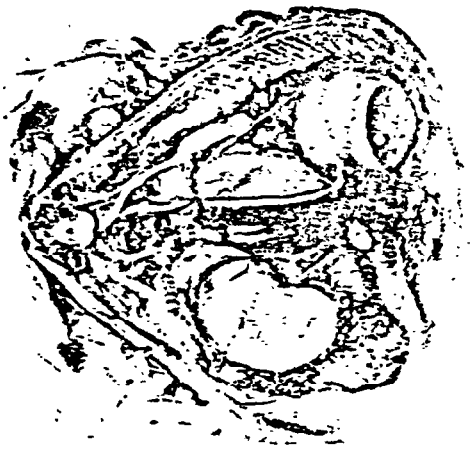
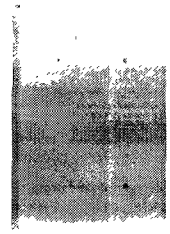


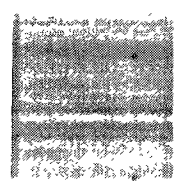
PLATE II-14 Kidney, testis and urinary bladder of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.



PLATEII-15 Kidney, ovary and uterus of control rabbit fetus; showing normally in the Wilson's section.

PLATEII-16 Kidney, ovary and uterus of fetus from the pregnant rabbit treated intraperitoneally with 5ml/kg of S-27; showing normally in the Wilson's section.





DEPARTMENT OF MICROBIOLOGY
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

December 1, 1977

Mr. Charles Lincoln
Delmont Laboratories, Inc.
P.O. Box AA, Swathmore
Pennsylvania 19081, U. S. A.

Dear Mr. Lincoln:

On behalf of the letter from Mrs. Emily H. Mudd of November 16, I am writing you in the hope that our data on the SPL obtained by our laboratory would be of some help for breaking the situation.

Last three years several members of our laboratory have engaged intensively in the research of SPL. Firstly, in order to test the effect of SPL, staphylococccus-sensitized mice were challenged with bacteria, fungi and viruses such as Listeria, Candida and herpes simplex virus, in combination with SPL treatment. However, the effect of staphylococcus itself was so great that the effect of SPL could not be detected. Therefore, considering that the SPL does activate macrophages provided the recipients were previously sensitized by staphylococcus (this is the situation in which the SPL is used in humans), we recently made the following experiments. Mice sensitized by Staphylococcus aureus 18Z strain were left for two months. These mice were found to be as susceptible to herpes simplex virus infection as untreated mice. After SPL treatment, however, these mice became resistant to herpes simplex virus infection (see attached report). The same kinds of experiments are under way by using bacteria and fungi.

Secondly, quantitation of the potency of SPL has also been of our interests. Since the principle of the effect of SPL is thought to be the activation of macrophages, we tried to find the sensitive system in which macrophages were used. We found that the effect of SPL could be quantitated by using suppressive effect of macrophages against murine ascitic tumor cells. As can be seen in attached report, good dose response was obtained between the SPL and its suppressive effect against ascitic tumor cells. This observation, in turn, will provide the support for the idea that the SPL is really a potent activator for macrophages.

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

I really hope this letter and the attached reports would be helpful for you.

Sincerely yours,

Kenji Takeya, M.D.
Professor of Bacteriology
President of Kyushu University

cc: Dr. Emily H. Mudd
Fujizoki Company

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

EFFECT OF SPL ON THE DEVELOPMENT OF SKIN LESION IN MICE AFTER INOCULATION WITH HERPES SIMPLEX VIRUS

Development of herpetic skin lesions produced in the midflank of mice after challenge with herpes simplex virus has been used as a model of skin response to the virus. In case of ICR mice, a vesicle appears at the site of injection 4 to 5 days after infection. Soon it changes to eruptic lesion remaining at the injected locus. By the 7th day a zoster-form lesion of eruption and necrosis develops on the inoculated side of the flank. Around this time the mice may die with involvement of the central nervous system.

With the use of this disease model, effect of SPL on the development of skin lesions in mice has been studied and the results obtained indicate that the SPL treatment has protective effect against herpes simplex virus inoculation in staphylococcus-sensitized mice.

Materials and Methods

Sensitization with staphylococcus: Staphylococcus aureus, strain 18Z, cultured in broth for 18 hr at 37C, was washed 3 times by centrifugation with phosphate buffered saline (PBS) and finally suspended in PBS to give 10^9 viable counts per ml. The suspension in 0.1 ml volume was inoculated intramuscularly once a week in one of the four extremities changing each time. For control studies PBS was inoculated instead of bacterial

suspension. Mice were used two months after the final injection of staphylococcus suspension.

SPL (staphylococcal phage lysate): SPL was supplied by Delmont Laboratories, Inc. A volume of 0.1 ml was inoculated intraperitoneally once a day. The treatment started two months after the last inoculation of staphylococcus-sensitization and continued for 14 days. For the control studies broth was inoculated in place of SPL.

Virus and challenge: Herpes simplex virus type 1, strain Hayashida, was used for challenge infection. The strain was isolated from a patient with active labial herpetic lesions in Vero cell cultures. After removing the hair manually over the midflank of ICR mice under a light anesthesia, a volume of 0.05 ml of the virus was injected intradermally by a 26 gauge needle. The virus challenge was done at 7th day of SPL (or broth for control) treatment.

Scoring the development of lesions: Development of the skin lesion was scored as follows; local vesicle 1, local eruption 3, local eruption with necrosis 4, scattered zoster-form lesion 6, continuous zoster-form eruption 8, continuous zoster-form eruption with severe necrosis 10.

Results

Susceptibility to herpes simplex virus of normal ICR mice (18Z-,SPL+) is shown in Fig. 1. A half of mice

developed severe zoster-form necrotic lesions. SPL-treatment without staphylococcus-sensitization (18Z-,SPL+) also had almost no effect, i.e., 5 out of 8 mice developed zoster-form lesions (Fig. 2). While the 18Z-sensitized mice (18Z+,SPL-) had a tendency of slightly resistant to herpes simplex virus infection without SPL treatment (Fig. 3), resistance of staphylococcus-sensitized and SPL-treated mice (18Z+,SPL+) had a strong tendency of resistance to herpes simplex virus infection (Fig. 4 and 5). Mean scores of lesions for each group are shown in Fig. 5.

Summary

SPL-treatment of ICR mice previously sensitized with Staphylococcus aureus was found to have protective effect against herpes simplex virus inoculation.

Legend for figures

Figs.1-4. Effect of SPL on the development of herpetic skin lesions in staphylococcus-sensitized mice. ICR mice were inoculated subcutaneously with Staphylococcus aureus, strain 18Z, 8 times with weekly intervals. Two months after the last inoculation of 18Z strain, the mice were inoculated with SPL with daily intervals for 14 days via intraperitoneal route. Seventh day after the first inoculation of SPL, mice were challenged intradermally with Hayashida strain of herpes simplex virus type 1. Development of local lesions and zoster-form lesion was scored thereafter. Control includes (1) without 18Z, without SPL treatment, (2) with 18Z, without SPL treatment, and (3) without 18Z, with SPL treatment.

Fig. 5. Mean scores of the herpetic skin lesions.

Fig. 1

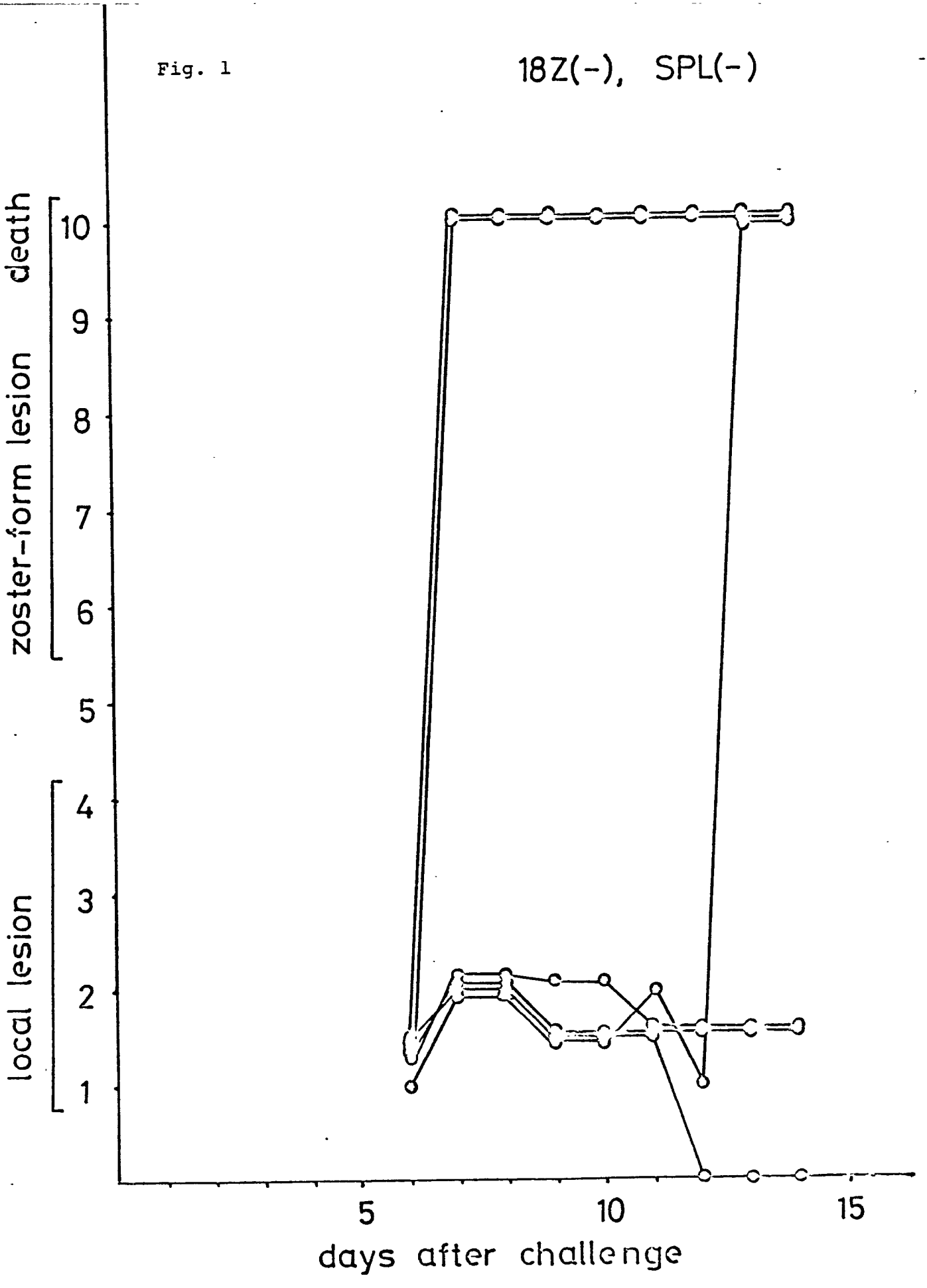

18Z(-), SPL(-)
zoster-form lesion death
10
9
8
7
6
5
local lesion
4
3
2
1
5
10
15
days after challenge

Fig. 2

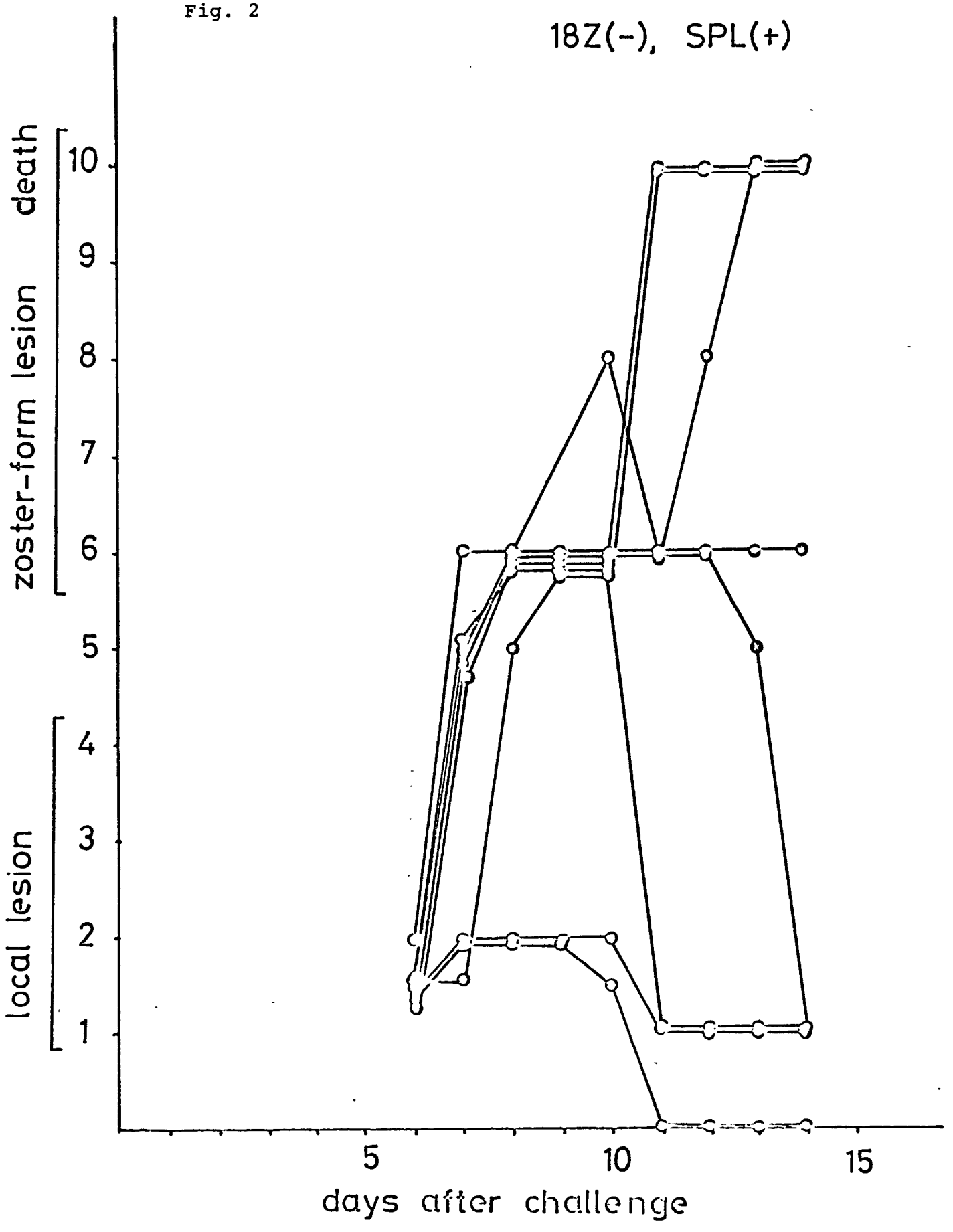

18Z(-), SPL(+)
zoster-form lesion
death
local lesion
10
9
8
7
6
5
4
3
2
1
5
10
15
days after challenge

Fig. 3

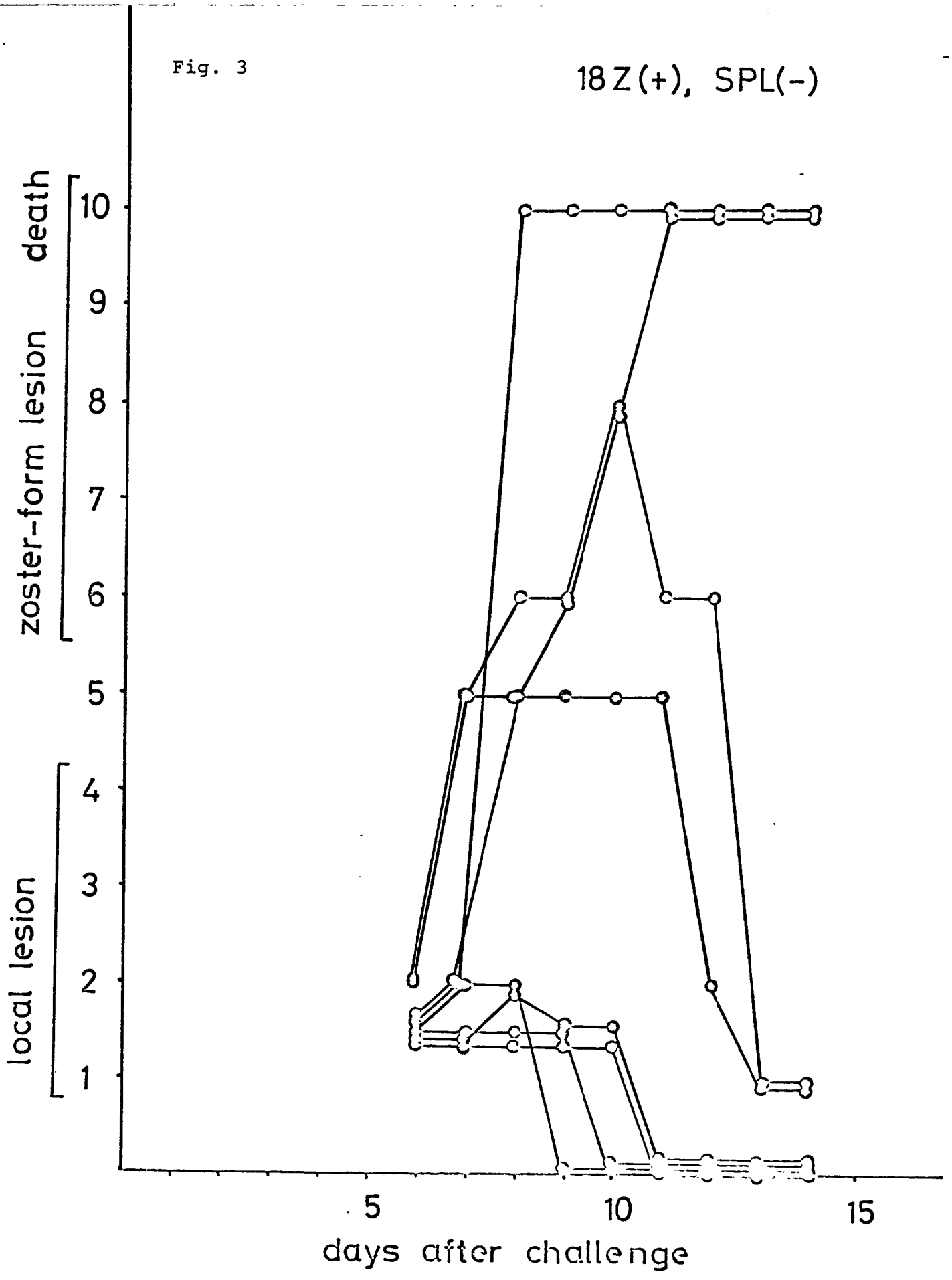

18 Z(+), SPL(-)
zoster-form lesion
death
10
9
8
7
6
5
local lesion
4
3
2
1
5
10
15
days after challenge

Fig. 4

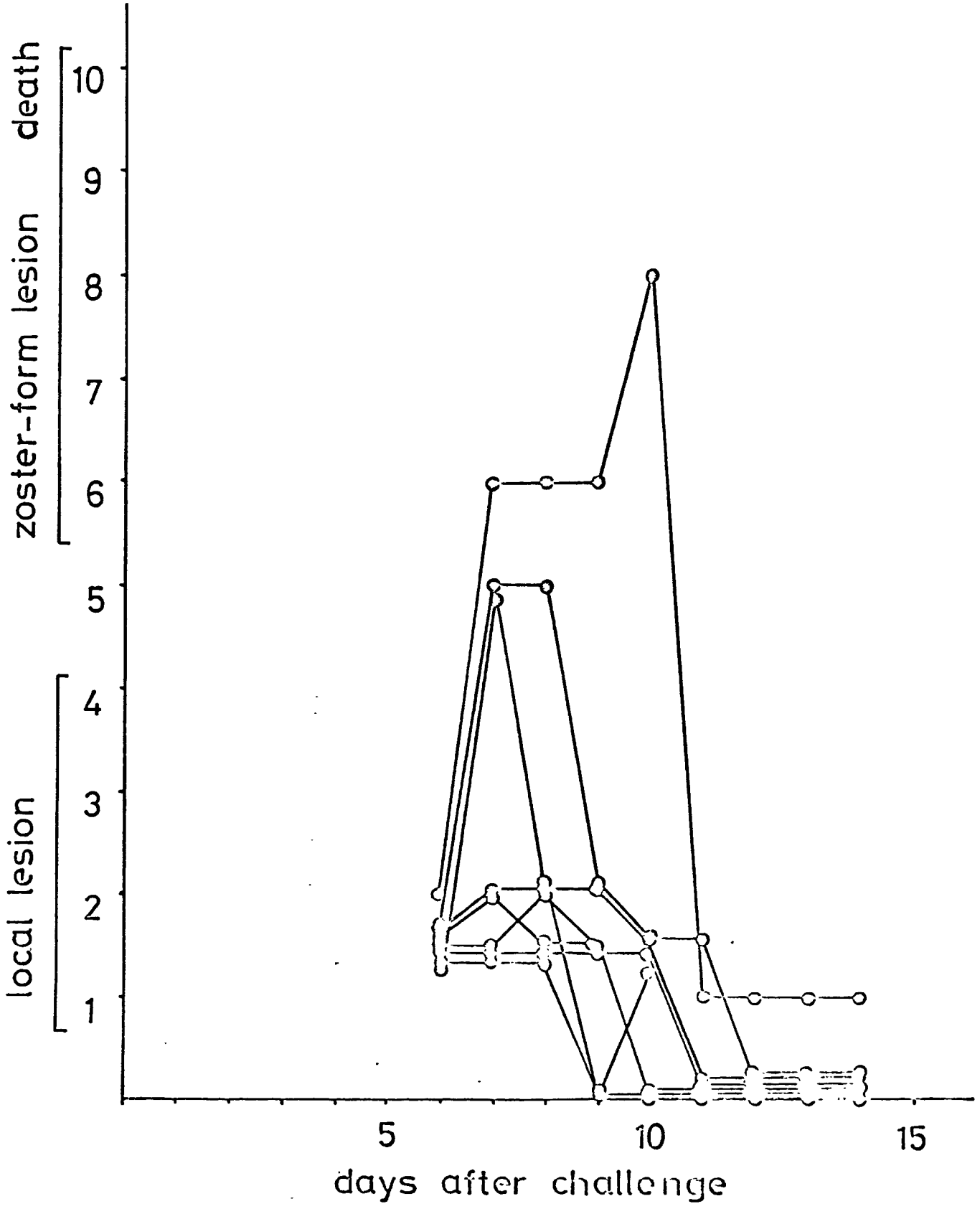
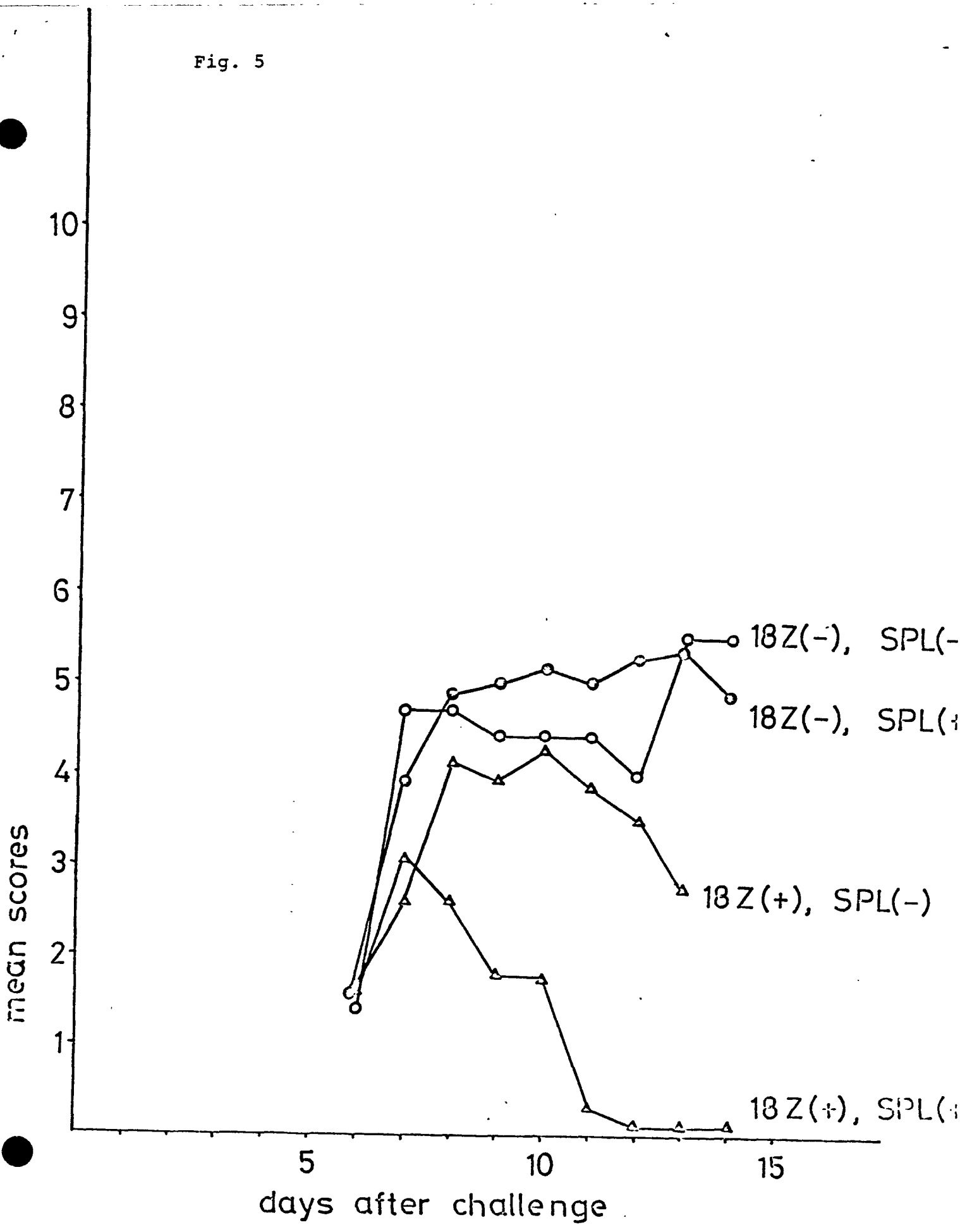

18 Z(+), SPL(+)
death
zoster-form lesion
local lesion
10
9
8
7
6
5
4
3
2
1
5
10
15
days after challenge

Fig. 5


10
9
8
7
6
5
4
3
2
1
mean scores
5
10
15
days after challenge
18Z(-), SPL(-
18Z(-), SPL(
18Z(+), SPL(-)
18Z(+), SPL(

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

CHEMOTACTIC ACCUMULATION OF MACROPHAGES IN THE PERITONEAL CAVITY AFTER INOCULATION OF SPL AND THEIR ANTITUMOR ACTIVITY

SPL has been presumed to activate macrophages and enhance the resistance to bacterial and fungal infections. Especially, SPL appears to exhibit such an effect on infections, when it is applied locally to the infected areas. Therefore, SPL may be able to accumulate macrophages locally and to activate them to give enhanced ability to kill microorganisms. The experiments were designed to analyze such abilities separately in mice.

Outbred ddY mice were used as hosts. Mice were injected subcutaneously with $1x10^8$ viable organisms of Staphylococcus aureus (18Z) once a week for 4 or 8 weeks for the pre-treatment. For the mesurement of chemotactic activity for macrophages, 0.1 ml of SPL was inoculated into the peritoneal cavity and peritoneal cells were counted at various intervals. For the assay of antitumor activity, $1x10^6$ viable cells of Sarcoma 180, an ascites tumor, were inoculated intraperitoneally and various amounts of SPL were inoculated intraperitoneally every day for 7 days after tumor inoculation. Volumes of ascites or total packed volumes of tumor cells were assesed on day 10.

Mice were pretreated with 18Z for 8 weeks and 0.1 ml of SPL was injected intraperitoneally 48 hr after the last inoculation of 18Z. Peritoneal cells were counted before the inoculation of SPL and 4,24 and 48 hr after the inoculation. When SPL was injected into normal controls,

the number of peritoneal cells increased slightly at 24 and 48 hr. When SPL was inoculated into mice pretreated with 18Z, the number of peritoneal cells increased rapidly and strikingly from 4 to 24 hr. (Fig.1)

Mice were pretreated with 18Z for 4 or 8 weeks and inoculated with tumor cells after the interval of 6 weeks. SPL was inoculated every day for 7 days from the day of tumor inoculation. When 1.0 ml of SPL was inoculated, tumor growth was inhibited completely in both groups(Tables 1 and 2). Definite effects were obtained with 0.3 ml of SPL in both groups. Suppressive effects were weak or negligible, when 0.1 ml of SPL was injected.

Summary SPL exhibited chemotactic activity for macrophages and antitumor activity on sarcoma 180 in mice pretreated with Staphylococcus aureus strain 18Z.

Fig. 1. Peritoneal cell number after intraperitoneal administeration of SPL (0.1 ml) in normal and 18Z-pretreated mice. (mean of 5 ± S.D.)

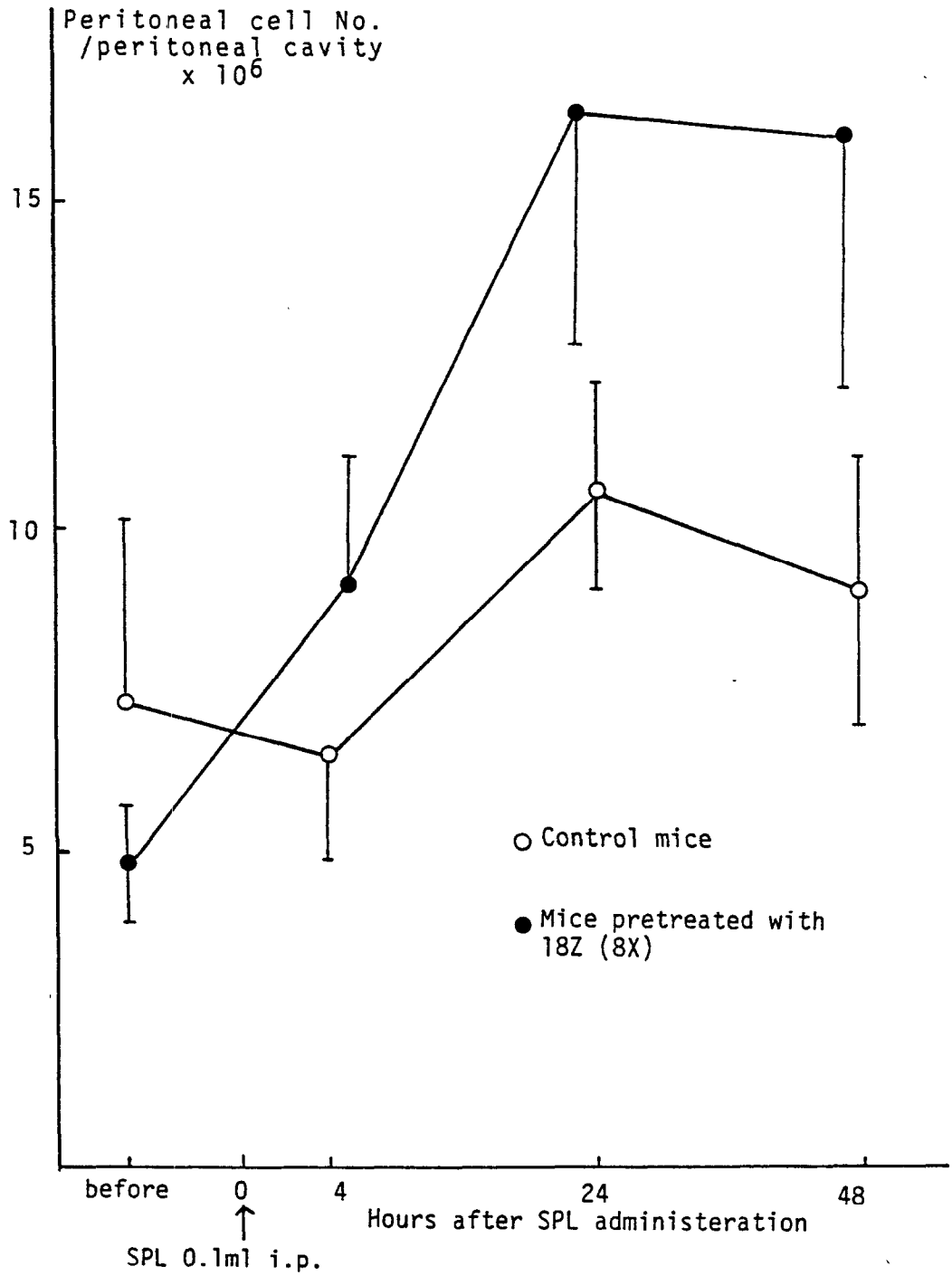


Table 1. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

S.aureus (18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid	Total packed cell volume	Cell growth (T/C)	Activity
	weeks	i.p.	i.p.	ml	ml	%	
1×10^8/body/week s.c. inj. x4	6	1×10^6	1.00	0	-	-	+++
			0.30	0.55	0.12	16.9	++
			0.10	1.08	0.35	49.3	+
			0	1.98	0.71	100	-

Table 2. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

S.aureus (18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid	Total packed cell volume	Cell growth (T/C)	Activity
	weeks	i.p.	i.p.	ml	ml	%	
			1.00	0	-	-	+++
			0.30	0.75	0.27	33.8	++
$1x10^8$/body/week s.c. inj. x 8	6	$1x10^6$	0.10	1.92	0.64	80.0	±
			0	2.30	0.80	100	-

FUJIZOKI PHARMACEUTICAL CO.,LTD.

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Tokyo, Dec. 28, 1977

Mr. Charles E. Lincoln, President
DELMONT LABORATORIES, INC.
P.O.Box AA, Swarthnore,
Pennysylvania 19081,
U.S.A.

JAN 4 1978

Dear Charlie:

For the purpose of presenting to forthcoming review by FDA, we herewith send you copies of the following datas and results of assessment.

1. Assessments and Studies of SPL
2. "S-27" Summary of Results of Tests Conducted at Fujizoki Pharmaceutical Research Division
3. Chemotactic Accumulation of Macrophages in the Peritoneal Cavity after Inoculation of SPL and Their Antitumor Activity.
4. Susceptibility of Staphylococcus aureus Clinical Isolates to Gratia Bacteriophage.
5. Influence of Staphage Lysates(SPL) on Immune Responses In Vitro. presented to The Fourteenth General Assembly The Central Regional Chapter of The Bacteriological Society of Japan
6. Immunopotentiator Activity of Staphage Lysate
7. Immunochemotherapy for Infections-------
   With particular reference to Staphage Lysate.

We hope these datas will be helpful to you, and wish you a Happy New Year.

Sincerely yours,

FUJIZOKI PHARMACEUTICAL CO., LTD

Yozo Matsubara, manager,
International Division

Assessments and Studies of SPL

I. Proposed Specifications, Tests and Assays (submitted to the Ministry of Public Welfare, June 9, 1977)

As stated in the attached paper, Summary of Results of Tests Conducted at Fuji-Zoki Pharmaceutical Research Division, dated May 27, 1977.

Supplementary Notes:

Staining.--- When examined microscopically with the gram stain, neither any gram-positive bacteria nor any gram-negative organisms are demonstrable in the product.

Sterility.--- When examined by the sterility tests, the product is (1) bacteria-free and (2) fungus-free.

Absence of abnormal Toxicity. --- Inject 5ml of SPL intraperitoneally into each of two guinea pigs weighing about 350gm and observe the animals for seven days after the injection: the animals show no discernible signs or symptoms of toxicity.

Histamine and Histamine-Like Substances. ---

Glass Containers. --- Glass containers for injection of SPL and (1) clear, colorless and free of any visible bubbles and (2) meet the requirements of the test for alkaline dissolution.

Insoluble Impurities. --- When examined with the naked eye holding the sample in a brightness of 1000 luces approx. directly below a white light source, the product is clear and contains no readily detectable, insoluble impurities.

II. Macrophage Chemotactic Test. (Mitsuyama, M., Miyake, T., Nomoto, K. and Taketani, K. from the Department of Bacteriology, Kyushu University School of Medicine) Confidential

Abstract:

Procedure of Test: Two groups of ten about 4-week-old ICR mice each were used. One group received single s.c. doses of 1×10^8 Staph. aureus 18Z cells weekly for a period of eight weeks for sensitization while the other group was kept untreated over the same period. Eighteen days after the final injection, the animals in these two groups were injected i.p. with 0.1ml of SPL and intraperitoneal macrophages of each animal were counted at 4, 24 and 48 hours after the eliciting injection.

Results: The animals given the eliciting dose of SPL after sensitization with Staph. aureus 18Z cells showed statistically significantly higher intraperitoneal macrophage counts, as compared to the control

group of normal untreated mice. The finding indicate that an eliciting i.p. dose of SPL gave rise to a significant intraperitoneal accumulation of macrophages in mice sensitized with Staph. aureus.

III. Test for Phage Activity. (Shigeno, N., Mitsuma, T. and Kojima, K. from the Junior College of Medical Technology and Nursing, affiliated with Niigata University. The 1977 Symposium on Staphylococci, Sept. 3, 1977, Okayama)

See the attached paper.

IV. Influence on Immune Responses In Vitro. (Mitsuma, T., Shigeno, N., Kojima, K. and Tanaka, Y. from the Junior College of Medical Technology and Nursing, affiliated with Niigata University, and from the Santo Hospital. The 14th Gen. Assembly of the Central Regional Chapter of the Jap. Soc. Bacteriol., June, 1977, Gifu; and the 41st East Japan Joint Meeting of the Jap. Soc. Dermatol., Sept. 24, 1977, Tokyo)

See the attached paper.

V. Immunopotentiator Activity. (Azuma, C., Tokuda, Y. and Shibata, T. from the Dept. of Dermatology, Tokyo College of Medicine. The 25th Gen. Assembly of the Jap. Soc. Chemotherapy, June 1977, Gifu, and the 41st East Japan Joint Meeting of the Jap. Soc. Dermatol., Sept. 24, 1977, Tokyo).

See the attached paper.

VI. Clinical Report. (Tsuda, S. and Minami, K. from the Dept. of Dermatol., Kurume University School of Medicine. MINOPHAGEN MEDICAL REVIEW, 21(5), 53-56, 1976)

See the attached paper.

VII. Controlled Double-Blind Trials. (To be concluded by the end of December 1977)

Subjects: Patients with multiple viral verrucosis.

Control drug: Broth (beef heart infusion broth) employed for the preparation of SPL.

Participating institutions: Tokyo Univ. (Dept. of Dermatol.), Kyushu Univ. (Dept. of Dermatol.), Defense Forces Univ. (Dept. of Dermatol.), Niigata Univ. (Dept. of Dermatol.), Ehime Univ. (Dept. of Pharmacol.), Tokyo Coll. Med. (Dept. of Dermatol.), Kurume Univ. (Dept. of Dermatol.) and Kansai Med. Coll. (Dept. of Dermatol.)

S - 27

SUMMARY OF RESULTS OF TESTS CONDUCTED AT FUJI-ZOKI PHARMACEUTICAL RESEARCH DIVISION

Institution: Division of Pharmaceutical Research, Fuji-Zoki Pharmaceutical Company, Ltd., Tokyo

Chief Investigator-in-Charge: Daiichi Watanabe,

Period of Testing: From April 12, 1977, till May 31, 1977

Laboratory Conditions of Testing: Room temperature, 20 - 24°C; relative humidity, 55-65%.

Test Samples: Lot Nos. 6090755, 6111462 and 6111463.

Nature and Description:

S-27 is a clear, colorless or slightly yellowish-brown liquid containing the filtrate from liquid culture of Staphylococcus aureus cells lyzed by specific bacteriophage.

Results

Lot No.	Description	Evaluation
6090755	This is a clear, colorless or slightly yellowish-brown liquid	Meets the requirements of the test
6111462	-do-	-do-
6111463	-do-	-do-

Hydrogen Ion Concentration:

Procedure of Test: The pH of S-27 was determined potentiometrically as directed in the Determination of Hydrogen Ion Concentration under General Test Procedures, the Biological Products Standards.

Results

Lot No.	Test No.	pH	Evaluation
6090755	1 2 3	7.45 7.46 7.45	Meets the requirements of the test
6111462	1 2 3	7.42 7.43 7.43	-do-
6111463	1 2 3	7.44 7.42 7.46	-do-

Staining:

Procedure of Test: The test was performed as directed in the Staining Test under General Test Procedures, the Biological Products Standards.

Results: All three Lots proved to meet the requirements of the staining test in all three repeated runs.

Sterility:

Procedure of Test: As directed in the Tests for Sterility (1) and (2) under General Test Procedures, the Biological Products Standards.

Results: Each Lot proved to meet the requirements of the tests for sterility in all three repeated runs.

Absence of Abnormal Toxicity:

Procedure of Test: As directed in the Test to Rule Out Abnormal Toxicity (1) under General Test Procedures, the Biological Products Standards.

Results: Each Lot proved to meet the requirements of the test to rule out abnormal toxicity (1) in all three repeated runs.

Weight Loss in Mice:

Procedure of Test: Inject 0.5ml of the product intraperitoneally into each of not less than five mice at approximately 5 weeks of age, and keep the mice under observation for 5 days after the injection: at the end of the 5-day observation the sum of the body weights of all mice exceeds that recorded on the day of injection, and the animals show no discernible symptoms of toxicity during and at the end of this period.

Results

Lot No.	Test No.	Total weight * on injection	Total weight 5 days later	Abnormality	Evaluation
6090755	1 2 3	98 gr. 96 89	111 gr. 109 104	Not recognized	Meets the requirements of the test
6111462	1 2 3	99 gr. 98 90	114 112 103	-do-	-do-
6111463	1 2 3	100 gr. 98 96	116 109 105	-do-	-do-

* Total weight of 5 mice

Pyrogen:

Procedure of Test: As directed in the Pyrogen Test under General Test Procedures, the Biological Products Standards, but using doses of 1.0ml of each test sample per kg of body weight of animals.

Results

Lot No.	Test No.	Total of animals involved	Total temperature of pyrogenetic animals	Evaluation
6090755	1 2 3	6 3 3	2.7°C 1.2 1.2	Meets the requirements of the test
6111462	1 2 3	3 3 3	1.2 1.0 1.2	-do-
6511463	1 2 3	3 3 3	1.2 1.1 1.2	-do-

Histamine and Histamine-Like Substances:

Procedure of Test: As directed in the Tests for Histamine under General Test Procedures, the Japanese Antibiotics Standards, but using doses of 0.02ml of test sample per kg of body weight of animals.

Results: Each Lot proved to meet the requirements of the test for histamine in all three repeated runs.

Anaphylatic Shock:

Procedure of Test: Select four guinea pigs each weighing between 350 and 500gm approx. Inject each animal intraperitoneally with 0.02ml of the test

sample q. 48 hours in a total of three doses for sensitization. Two and three weeks after the final sensitizing dose test the animals for anaphylactic shock by single intravenous injection of 0.2ml of the same test sample, using two animals each: the animals show no discernible signs or symptoms of shock.

Results: Each Lot was found to meet the requirements of the test for anaphylactic shock in all three repeated runs.

Glass Containers:

Procedure of Test: As directed in the Test of Glass Containers for Injection under General Test Procedures, the Japanese Pharmacopeia.

Results:

Lot No.	Test No.	(1)	(3) Procedure I*		Evaluation
			Quantity of Sample Taken	Titer	
6090755	1	Clear, colorless and no bubles	5.0010(g)	0.06(ml)	Meets the requirements of the test
	2	- " -	5.0006	0.05	
	3	- " -	5.0005	0.05	
6111462	1	- " -	5.0005	0.05	"
	2	- " -	5.0008	0.05	
	3	- " -	5.0005	0.05	
6111463	1	- " -	5.0005	0.06	"
	2	- " -	5.0006	0.05	
	3	- " -	5.0005	0.05	

* Factor for 0.02N sulfuric acid = 1.009

Insoluble Impurities:

Procedure of Test: As directed in the Item (11) under Injections, the General Notices, J.P.

Results: Each Lot proved to meet the requirements under Injections (11) in all three repeated runs.

Assay:

Carry out the assay by the phage plaque-forming unit (PFU) counting technique on plates of Trypticase soy agar (TSA) seeded with Staphylococcus aureus.

Materials: Use Trypticase soy broth (TSB) as the diluent for the test sample S-27, and a 3-hour TSB culture (31°C) of Staphylococcus aureus strain 3A as the reference organism.

Assay Results: Each ml of the test sample contains not less than 5×10^7 and not more than 5×10^8 PFU of staphylococcal bacteriophage.

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF MEDICINE, KYUSHU UNIVERSITY
FUKUOKA, 812 JAPAN

CHEMOTACTIC ACCUMULATION OF MACROPHAGES IN THE PERITONEAL CAVITY AFTER INOCULATION OF SPL AND THEIR ANTITUMOR ACTIVITY

SPL has been presumed to activate macrophages and enhance the resistance to bacterial and fungal infections. Especially, SPL appears to exhibit such an effect on infections, when it is applied locally to the infected areas. Therefore, SPL may be able to accumulate macrophages locally and to activate them to give enhanced ability to kill microorganisms. The experiments were designed to analyze such abilities separately in mice.

Outbred ddY mice were used as hosts. Mice were injected subcutaneously with $1x10^8$ viable organisms of Staphylococcus aureus (18Z) once a week for 4 or 8 weeks for the pre-treatment. For the mesurement of chemotactic activity for macrophages, 0.1 ml of SPL was inoculated into the peritoneal cavity and peritoneal cells were counted at various intervals. For the assay of antitumor activity, $1x10^6$ viable cells of Sarcoma 180, an ascites tumor, were inoculated intraperitoneally and various amounts of SPL were inoculated intraperitoneally every day for 7 days after tumor inoculation. Volumes of ascites or total packed volumes of tumor cells were assesed on day 10.

Mice were pretreated with 18Z for 8 weeks and 0.1 ml of SPL was injected intraperitoneally 48 hr after the last inoculation of 18Z. Peritoneal cells were counted before the inoculation of SPL and 4,24 and 48 hr after the inoculation. When SPL was injected into normal controls,

the number of peritoneal cells increased slightly at 24 and 48 hr. When SPL was inoculated into mice pretreated with 18Z, the number of peritoneal cells increased rapidly and strikingly from 4 to 24 hr. (Fig.1)

Mice were pretreated with 18Z for 4 or 8 weeks and inoculated with tumor cells after the interval of 6 weeks. SPL was inoculated every day for 7 days from the day of tumor inoculation. When 1.0 ml of SPL was inoculated, tumor growth was inhibited completely in both groups(Tables 1 and 2). Definite effects were obtained with 0.3 ml of SPL in both groups. Suppressive effects were weak or negligible, when 0.1 ml of SPL was injected.

Summary SPL exhibited chemotactic activity for macrophages and antitumor activity on sarcoma 180 in mice pretreated with Staphylococcus aureus strain 18Z.

Fig. 1. Peritoneal cell number after intraperitoneal administeration of SPL (0.1 ml) in normal and 18Z-pretreated mice. (mean of 5 ± S.D.)

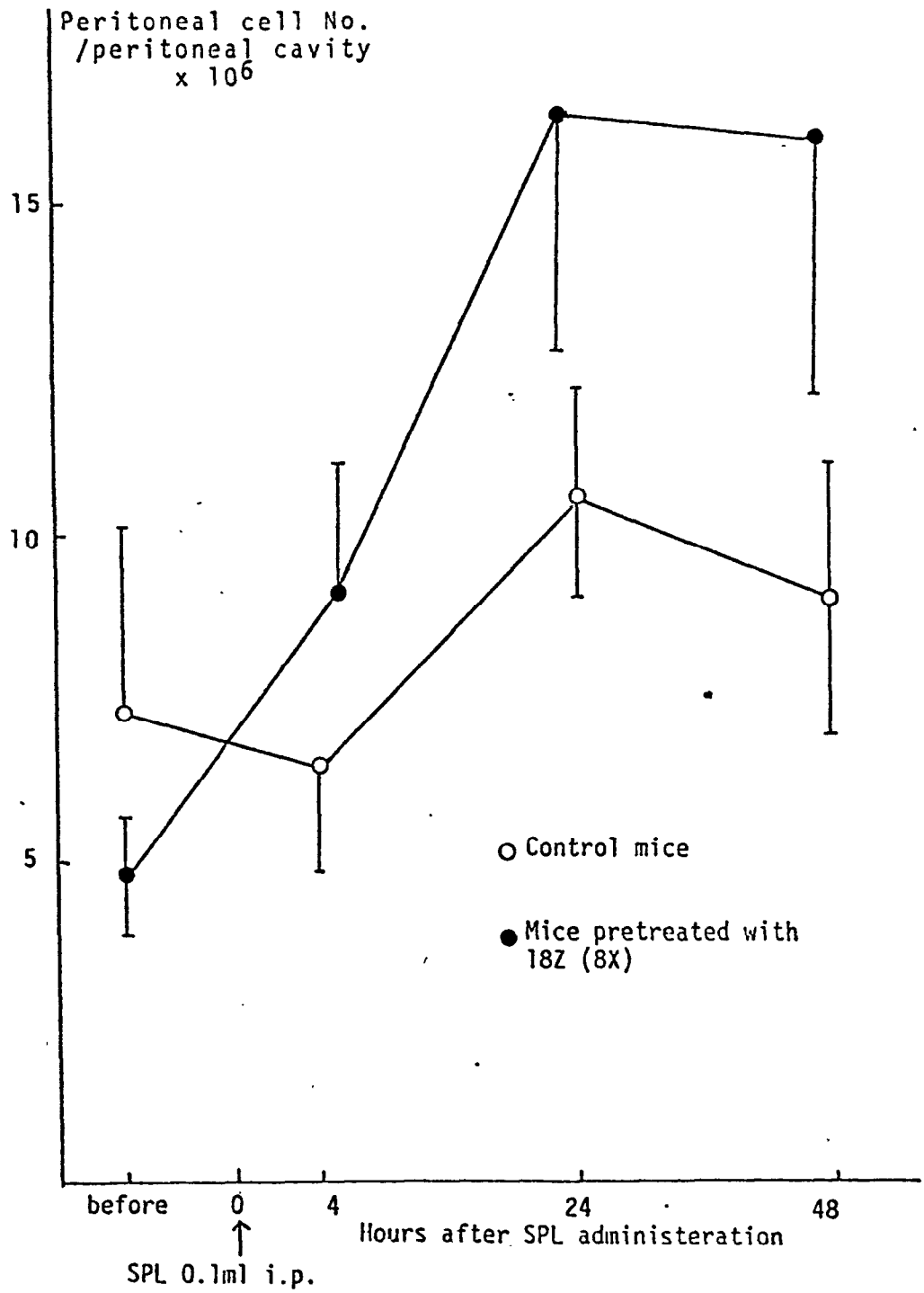

Table 1. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

S.aureus (18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid	Total packed cell volume	Cell growth (T/C)	Activity
	weeks	i.p.	i.p.	ml	ml	%	
			1.00	0	-	-	+++
$1x10^8$/body/week s.c. inj. x4	6	$1x10^6$	0.30	0.55	0.12	16.9	++
			0.10	1.08	0.35	49.3	+
			0	1.98	0.71	100	-

Table 2. Antitumor activity of SPL against Sarcoma 180 in ddY mice.

S.aureus (18Z)	Interval	Sarcoma 180 implantation (Cell No./body)	SPL (ml/body)	Volume of ascitic fluid	Total packed cell volume	Cell growth (T/C)	Activity
	weeks	i.p.	i.p.	ml	ml	%	
			1.00	0	-	-	+++
			0.30	0.75	0.27	33.8	++
$1x10^8$/body/week s.c. inj. x 8	6	$1x10^6$	0.10	1.92	0.64	80.0	±
			0	2.30	0.80	100	-

Susceptibility of Staphylococcus aureus Clinical Isolates to Gratia Bacteriophage

Shigeno, N., Mitsuma, T. and Kojima, K.

Junir College of Medical Technology and Nursing affiliated with Niigata University

Summary:

(1) Of a total of 466 Staphylococcus aureus isolates from various clinical specimens studied, 201 strains (45.1%) were found susceptible to SPL.

(2) Isolates from the otorrhea, in particular, were very frequently susceptible to the phage (43 out of 54 strains, or 79.6%).

(3) Isolates from the sputum showed a relatively low rate of susceptibility, 41 out of 118 strains or 34.7%.

(4) Organisms isolated from the nasopharynx were almost as susceptible as those from the pus, the rates being 78/181 (43.1%) and 29/68 (42.6%), respectively.

References:

1) Gratia, A., Proc, Soc, Exp, Biol. Med. 18, 217 (1921).

2) Larkum, N.W., J. Inf. Dis. 45, 34 (1929).

3) Smith, P. B. and Mudd. S., Proc. Soc. Exp. Biol. Med. 134, 225 (1970).

Table 1. Susceptibility to SPL of Staph. aureus clinical isolates.

		Naso-pharynx	Suptum	Otor-rhea	Pus	Urine	Other	Total No. Strains
Susceptible		78 (43.1)	41 (34.7)	43 (79.6)	29 (42.6)	7 (41.2)	3 (37.5)	201 (45.1)
Degree of susceptibility	+++	53 (29.3)	26 (22.0)	22 (40.7)	16 (23.5)	5 (29.4)	2 (25.0)	124 (27.8)
	++	13 (7.2)	6 (5.1)	10 (18.5)	5 (7.4)	2 (11.8)	0	36 (8.1)
	+	12 (6.6)	9 (7.6)	11 (20.4)	8 (11.8)	0	1 (12.5)	41 (9.2)
Insusceptible		103 (56.9)	77 (65.3)	11 (20.4)	39 (57.4)	10 (58.8)	5 (62.5)	245 (54.9)
Total No. Strains		181 (100)	118 (100)	54 (100)	68 (100)	17 (100)	8 (100)	446 (100)

* Figures represent the numbers of isolates, and those in parentheses the corresponding percentages.

The Fourteenth General Assembly

THE CENTRAL REGIONAL CHAPTER OF THE

BACTERIOLOGICAL SOCIETY OF JAPAN

Abstracts of Presentations

Director, Central Chapter: Dr. Sadao Miyamura, professor, Niigata University School of Medicine

Charman of the Assembly: Dr. Wataru Kondo, professor, Niigata University School of Dentistry

Session: From 14:25 to 17:45, Oct. 29 (Sat.), 1977
From 09:00 to 14:00, Oct. 30 (Sun.), 1977

Place: Lecture Hall, Niigata University School of Medicine, 757 Ichibancho, Asahimachidori, Niigata City

14. Influence of Staphage Lysates (SPL) on Immune Responses In Vitro

Mitsuma, T,* Shigeno, N.,* Kojima, K.* and Tanaka, M.** (* Junior College of Medical Technology and Nursing affiliated with Niigata University and ** Santo Hospital)

Objective:

Staphage Lysates (Delmont Laboratories Inc., U.S.A.) (SPL) is the whole product from lysis of Staphylococcus aureus cells by specific bacteriophages and, therefore, not only holds the active phage in it but also contains bacterial cellular components as well as the constituents of culture medium. It has initially been introduced for use as a therapeutic agent against Staphylococcus aureus infection and, recently, has been acquiring importance on account of its non-specific immunostimulant property. This report describes the results of an in vitro study we conducted to investigate the effect of SPL on antibody production.

Materials and Methods:

The study was carried out using DK1 mice and sheep erythrocytes (SRBC) as antigen. Normal mouse splenic cells (2×10^7 cells per tube) were cultured with

SRBC in Marbrook tubes in a CO_2 incubator at 37°C. After four days of incubation, the cultures were examined for numbers of antibody-producing cells by the plaque-forming cell (PFC) counting according to the Mishell-Dutton method. Immediately prior to incubation various concentrations of SPL were added to the cultures to assess their effect on the PFC assay in comparison with SPL-free controls.

Results and Discussion:

A significant increase in the number of antibody-producing cells was evident in cultures containing SPL; the cultures to which SPL had been added at a final concentration of 10% showed an approximately three-fold increase of PFC (1260±28/tube), compared to the SPL-free control (PFC: 400±16/tube). With the decrease in the concentration of SPL added to the culture, the PFC diminished progressively to approach the control level.

An additional set of experiments was performed in the same manner and with the same reagents as in the foregoing experiment but using, in place of the normal mouse splenic cells, a splenic cell fraction except T cells eliminated by treating normal mouse spleen cells with anti-C_3H brain rabbit (anti-Thy) serum and complement. There was no appreciable effect of SPL on these cells; the cultures containing SPL showed essentially the same PFC

counts as the SPL control. The finding does not suggest that SPL has the ability to act directly upon antibody-producing cells to facilitate their nonspecific division.

Further studies to clarify the mechanisms of action of SPL, particularly in these respects, are in progress.

TABLE Effect of SPL on the in vitro Anti-SRBC response of mouse spleen cells

Concentration of SPL (%)	In vitro Immunization $\overline{C}$ SRBC	PFC/Culture	
		Treatment $\overline{C}$ -	Anti-Thy+C +
-	-	128 ± 40	156 ± 4
-	+	400 ± 16	N.D.
10	+	1260 ± 28	192
1	+	600 ± 24	216 ± 32
0.1	+	456	164 ± 20

221. Immunopotiator Activity of Staphage Lysate (Mudd)

Azuma, C., Tokuda, Y. and Shibata, T.
Department of Dermatology, Tokyo
College of Medicine, Tokyo

Staphage lysate (Mudd), referred hereinafter to as SPL, is a staphylococcal phage and its extensive studies of Prof. Mudd and his associates have demonstrated enhancement of resistance to infections in animals treated with this preparation. The underlying immunologic mechanism, nevertheless, is not clearly known as yet. This presentation summarizes the results of our recent study leading us to conclude that SPL has the property of acting as an immunopotentiator.

1) A series of patients with collagen diseases and other immune deficiency syndrome received subcutaneous doses of 10^7 to 10^8 SPL, each course consisting of ten doses injected q. 48 hours. There was clinical evidence of significantly increased defensive capacity against infection in these cases. Enhanced immune responsiveness was also observed in the PPD skin test.

2) In the treated series of patients, increase in bactericidal activity of neutrophils appeared to

parallel the enhancement of cutaneous response to PPD.

These findings indicate potentiation of the function of peripheral neutrophile leukocytes by SPL.

3) Experiments were performed to assess the effect of SPL in enhancing the resisting power of the host against infection, compared to various other antigenic sensitization in terms of minimal pus-forming dosis, with the results summarized in the table shown below. As can be seen, a greater degree of increase in the host's resistance to infections was obtained by sensitization with live staphylococci than by that with SPL alone.

Minimal Pus-forming Dosis (72h - 96h)

	Antigen Staph. aur.		Bacterial counts			
	Imm. R	Del. R	2800×10^4	1400×10^4	700×10^4	350×10^4
Staph. sensitization	(-)	(++)	? ++	derma-titis	derma-titis	derma-titis
Staphage lysate (Mudd)	(++)	(-)	+++	+++	+	±
Staph. infection with croton oil dermatitis			++	+ - ±	-	-
BCG sensitization	(-)	(-)	++	++	+	±
DNCB sensitization	(-)	(-)	++	++	+	±
Cont.	(-)	(-)	++	++	+	±

* Sensitization with live cells

4) Significant enhancement of the resistibility to infections was obtained by inoculation with SPL in rabbits and mice previously sensitized with live bacterial cells (Staph. aureus strain 209P). Furthermore, peritoneal macrophages from these animals showed increased bactericidal activity in vitro.

The data indicate that SPL acts as an immunopotentiator on the lymphocyte-macrophage-neutrophil system.

The 25th General Assembly of the Japanese Society of Chemotherapy, June 1977.

Immunochemotherapy for Infections -----
With Particular Reference to Staphage Lysate

Shingo Tsuda and Kikuo Minami
Department of Dermatology, Kurume University
School of Medicine, Kurume, Fukuoka Prefecture

Apart from the present subject immune deviation, I would like to add some comment on treatment of such severe, intractable staphylococcal infections of the skin. It will be concerned with staphage lysate, or SPL, supplied from Dr. Taketani of the Department of Bacteriology, Kyushu University, for clinical trials. The preparation is the product from lysis of Staphyococcus aureus by staphylococcus bacteriophage and, consequently, totally represents the antigenic components of the organism as illustrated in Table 7. In the United States, it has been clinically tried in more than 3,000 cases by Mudd and coworkers and reported to have proven effective in chronic refractory cases of staphylococcal infections. Through the clinical experience with SPL in these over 3,000 patients it has been ascertained that SPL is non-irritant and non-toxic and has no sensitizing effect in man. A pattern of erythematous reactions of considerable

interest has been observed in the skin test with SPL performed on patients with staphylococcal infections (Fig. 13). That is, in patients receiving intradermal injections of SPL (0.1ml) at weekly intervals, both the immediate and delayed local reactions were pronounced after the first intradermal injection and, thereafter, the local erythematous reaction diminished progressively in intensity and at the same time there occurred a progressive symptomatic improvement. A similar phenomenon has also been observed at our clinic.

We performed clinical trials of SPL in the cases shown in Table 8. The patients were treated with SPL alone and each patient was assessed as to degree of clinical improvement to evaluate effectiveness of the medication. Most of the patients studied had chronic intractable staphylococcal infections while the case material also included some patients with viral diseases. The treatment was considerably effective although no conclusive statement can be made here because of the relatively small series studied.

I would like to briefly describe two of these cases. A 62-year-old male had lesions of roentgen ulcer with secondary infection in the right dorsum pedis and interdigital regions, with so pronounced local edematous swelling as to cause difficulty in walking. The patient had been

treated elsewhere with antibiotics and other medicaments over the past few years, and, as the previous treatment failed to produce any significant improvement, he was begun on SPL. Figure 14 is a photograph of the affected area of the right foot taken on the first examination at our clinic.

Figure 15 shows the exanthems of the same area about two months after the start of SPL therapy, by which time he received a total dose of 9ml of the drug. The patient became completely relieved of edema and swelling and also considerably relieved from difficulty in walking.

The cutaneous lesion about 6 months of SLP therapy with a total dose of 16.5ml is shown in Figure 16. By this period the secondary infection had subsided almost completely and the patient became capable of walking as usual.

Another male patient, aged 63 years, was initially treated with oral and parenteral antibiotics along with ointments containing antibiotics since Staphylococcus epidermidis was isolated from pustules (Fig. 17). As no trend to improvement was noted in a few weeks of the antibiotic therapy re-examination was made and cultures disclosed Candida parakrusei and Trichophyton rubrun; the case was diagnosed as sycosis parasitaria.

Figure 18 is photograph of the same case, showing

the region two months after the first examination. The patient received SPL in subcutaneous doses of 0.5ml and intradermal doses of 0.6ml and a topical antitrichophytic preparation. Whilst the mechanisms whereby SPL produces such clinical improvement as yet are not clear, Mudd et al. have inferred that the administration of SPL elicites delayed hypersensitivity which has been previously induced to Staphylococcus aureus, thereby leading to activation of macrophages with consequent specific or nonspecific clinical effects.

From the analysis of the host's immune responses to staphylococci, it would seem rational to speculate that the disease state of severe intractable infection represents a condition which may be referred to as immune deviations. It is considered to be of profound significance that the phenomenon was observed not in such relatively rare diseases as leprosy and leishmaniasis but in staphylococcal infections of the skin which are commonly encountered. It has long been questioned whether cell-mediated immunity might have any significance in the host's defense mechanism against staphylococcal infection, but the results of the present analysis seem to reconfirm its importance. It follows that, in treating the host with intractable infection consequent to deviations of immune

response, chemotherapy alone does not suffice but immuno-chemotherapy for the infection should be undertaken as in immunochemotherapy for cancer.

The author is gratefully indebted to Prof. Takeya, Department of Bacteriology, Kyushu University School of Medicine, for the generous supply of SPL preparation. Acknowledgement is also made to Dr. Nomoto, assistant professor of medical bacteriology, Kyushu University, for his constant interest and guidance in this investigation.

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Table 7. Staphage Lysate (SPL) is the Complete Representation of the Antigenic Components of Staphylococcus aureus.

SPL contains the metabolites of the Staphylococci.

SPL contains both the heat stable and heat labile antigenic fractions, plus the intra- and extracellular enzymes of the Staphylococci.

SPL contains the solubilized products of the cell wall and the protein contents of the lysed Staphylococci.

SPL contains the active bacteriophage which produced the lysis of the Staphylococci.

SPL is a laboratory-fresh product, wholly free of preservatives or other denaturants.

Table 8

Nature of infection	Clinical results			
	Total	Excellent	Greatly improved	Unimproved
Folliculitis	2	1	1	
Furuncle	3	1	2	
Furunculosis	2		1	1
Ulcer due to radiation + Secondary infection	1	1		
Acne pustulosa	3	1	2	
Sycosis parasitaria	1	1		
Herpes zoster	6	3	2	1
Pustulosis palmo-plantaris	2			2

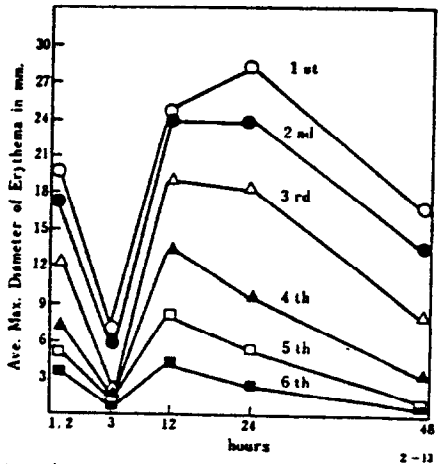


Fig.13 Erythematous reactions over a 2-day period in patients receiving six intradermal injections of SPL (0.1 ml) at weekly intervals.
(Mudd. S. : J. Reticuloend. Soc., 8, 1970)

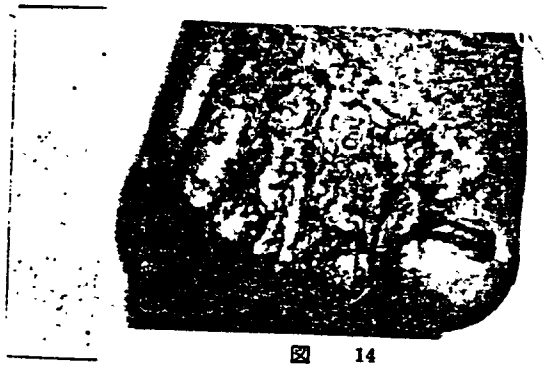


図 14

Fig. 14

図 15

Fig. 15

図 16

Fig. 16

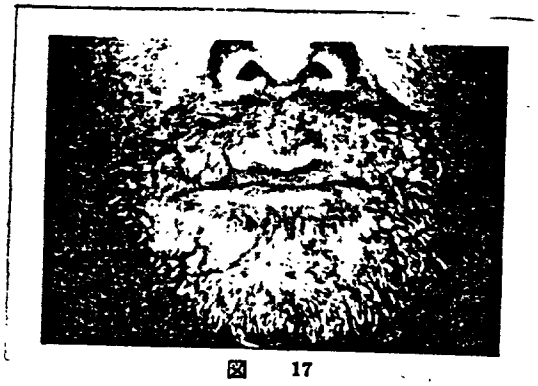


図 17

Fig. 17

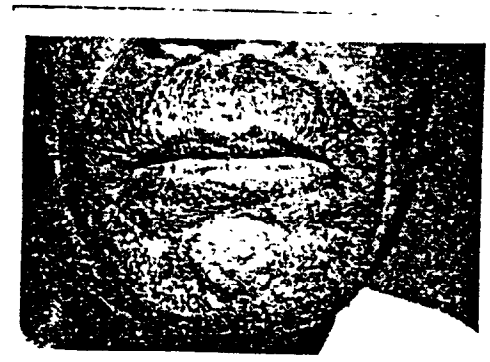
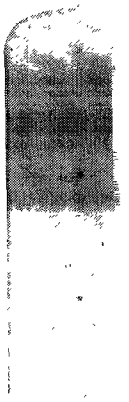
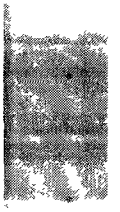


図 18

Fig. 18





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SHORT and LONG TERM SURVEILLANCE OF RECIPIENTS OF STAPHAGE LYSATE THERAPY

from the practice of ARTHUR G. BAKER, M.D., F.A.C.A.

I. Method of obtaining subjects:

(a) Fifty or more consecutive patients who started on SPL therapy in 1972.

(b) Twenty patients who have received SPL therapy for more than ten years.

II. Case Reports:

(a) Patients who responded to SPL therapy, but no longer receive SPL.

(b) Patients who continue to receive SPL

(c) Patients who discontinued therapy, and reason for dropping out.

(d) Present condition of above patients.

Patient Treated with Staphage Lysate Therapy. (SPL)

Case Report

Patient No. _____ Male_____ Age_____
Female_____ Occupation_____

Diagnosis:

1. Initial condition for which SPL was administered.

2. Concurrent diseases for which SPL was not administered.

3. Patient treated with SPL

From:_____ to _____
From _____ to _____
From _____ to _____

4. Routes of Administration.

Subcut._____ Intranasal _____ Topical _____Other_____

5. Dosage by each route:

6. Approximate number of treatments:

7. Patient's response:

8. Present condition:

(Signed)_____
Date _____